

nature

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Is the dual-support system really immutable?

Is a time of no-growth one for holding the line and stabilising institutions against erosive forces, or is it one for shaking up the system, bringing in reforms and generally ensuring that if and when growth returns a new structure is there to take advantage of it? This is a frequent question, in one of its many guises, which was asked last week by Mr Gerry Fowler, Minister of State at the Department of Education and Science when addressing heads of university chemistry departments on future government policy for scientific research. And, as if to underline the no-growth theme, later in the week the government announced that although the universities' recurrent grant for day-to-day expenditure would rise 11% to £581 million in 1976-7, this figure would be a cash limit, not to be supplemented (as in the past) for additional academic salary rises.

Mr Fowler gave the impression that his department had a fairly open mind on some of the major policy issues, or at least that views were still welcome on such matters as the customer-contractor principle, the quinquennial system and the dual-support system for university research. He also resurrected the question that many universities have been studiously avoiding for a long time: how to recognise more effectively the university teacher who is not inclined to do research as his primary occupation.

In the past year the dual-support system—the concept that one organisation gives general grants to

support universities and provide a 'well-found' base for another organisation to support specific research projects—has been given some fairly widespread support. A Commons select committee has positively been angling for criticism of the system but has found relatively little: such criticism as there has been has tended to be about alleged shabby treatment by one arm or the other rather than about the concept as such. So should we all settle back and let the system run on as it is?

There seem to be two defects which have not been adequately discussed yet. The first was raised by Mr Fowler himself—the university teacher. The dual-support system should offer every encouragement to the teacher who wishes only to take advantage of the well-found base and not to go after research council money. But somehow academics have got it into their heads that the badge of office is incomplete without a research grant. Second, although the system should be a support to the teacher, it can very easily be a shelter to the mediocre whose proposals to research council are regularly turned down. There has recently been much talk, not all of it malevolent, about eliminating mediocre people, departments or even universities. At present the machinery for so doing would be cumbersome in the extreme. One prop can be knocked out, but hardly both simultaneously. Would any modification of the present system of financial support provide a more challenging environment without making the rôle of the teacher even more insecure?

Don't despise the miracle

MR JOHN FAGAN of Glasgow, suffering from a stomach cancer, was on the verge of death. The doctors had given him up, and fully expected him to die within a day or two. But his fellow-parishioners from the Roman Catholic church of The Blessed John Ogilvie were busy praying to John Ogilvie, who had been executed in 1615 for refusing to recognise the supremacy of King James I on spiritual matters. Mr Fagan survived. Nobody could find any physical cause for the dramatic turn-around in his health, and now the Roman Catholic Church has accepted the cure as miraculous and set John Ogilvie on the way to becoming a saint. At which you might expect us to sneer.

After all, there are plenty of cases where remission has occurred inexplicably; one-in-a-million chances do turn up once every million times. And what about all those cases, not given publicity by the church, where saints have been prayed to and have failed to deliver a cure? Surely in a world of scientific objectivity there

can be no room for the miracle cure; the church is capitalising on rare and random phenomena.

This laboratory-bench view shows well the limited horizons that we may impose on ourselves. For we cannot yet cure cancer, least of all of the advanced sort that Mr Fagan was afflicted with, in spite of the hundreds of thousands of man-years devoted to scientific study of the subject; does that allow us to pass judgement on others who are also groping, but in entirely different territory? It does not.

It might if we were successful in curing cancer and if the church were actively dissuading its members from accepting medical treatment as a substitute for prayer to the saints. It might if the church were, as do many purveyors of quack cures, to claim a high success rate and to demand a large fee. Until then it were better that saints and scientists co-exist and be thankful for any small mercy, whether it be Mr Fagan's cure or a flash of insight accorded to some cancer researcher.





Picture: Mary Evans

Frozen mineral waters

Allan Piper looks at the obstacles to exploitation of the vast and varied resources of Antarctica

ASSESSMENTS of the economic potential of Antarctica involve issues stretching far beyond the obvious questions of how much and what sort of wealth might be there for the taking. With conditions on the continent hostile and often unpredictable, the successful operation of man, machines and extraction plant is hampered by extremes of climatic behaviour. Huge costs are imposed by Antarctica's extreme distances from the major centres of the commercial world. From the tip of the Antarctic Peninsula the journey to Cape Town is over 4,000 miles, and distances to the principal ports of the Northern Hemisphere exceed 7,000 miles. Moreover, during the winter months much of the continent is inaccessible by sea.

But there are other problems too, problems of particular relevance to any future sessions of the Law of the Sea Conference. The legal position in Antarctica is far from certain because nobody has been able to decide to whom the continent should belong. Territorial sovereignty is, and always has been, very much an open question, and the political jealousies run very deep. It is easy to see why. The history of Antarctica is characterised by diplomatic confusion. Conflicting claims for its discovery have never been satisfactorily settled, and in any case none of them was supported with the conventional backing of permanent occupation until over 120 years after the continent first became known around 1820.

What is certain is that a drive to satisfy commercial demands lay behind the eventual discovery of Antarctica, probably by the sealers who by the 1820s had penetrated southern waters as far as the South Shetland Islands bordering the Antarctic Peninsula; over the following century the whaling industry grew to become Antarctica's principal resource in the wake of brutal

over-exploitation of the native fur seals and elephant seals. By 1959, when the Antarctic Treaty was signed, annual whale catches had risen to over 40,000.

Throughout the expansion of the whaling industry, however, commercial interest in Antarctica moved steadily back into second place as the continent became increasingly prized for its prestige value and potential strategic advantages. By the close of World War II national rivalries were running high. Seven countries—Argentina, Australia, Britain, Chile, France, Japan, and Norway—had asserted territorial claims and backed them up with permanent bases.

With 80% of Antarctica neatly divided into pie-slices the geographical borders became more satisfactorily defined than the diplomatic boundaries. Along with the seven existing claimants both the USA and the Soviet Union had established presences on the continent, but neither recognised other territorial claims and neither asserted any. Tensions between Argentina, Britain and Chile, whose claims partially overlap, had stretched to breaking point.

Taken in this context, the signing of the Antarctic Treaty in 1959 must be seen as a major diplomatic achievement. Belgium, New Zealand and South Africa joined the other nine states in agreeing to maintain Antarctica free of military presence, and to hold all territorial claims in abeyance, so that just as no new claim can be asserted, so none is recognised and none challenged.

It would be too charitable to suggest that none of the signatories had half an eye to the commercial potential of Antarctica when the treaty was signed, but nonetheless mineral exploration and exploitation rights are not mentioned—the feeling seemed to be that such a recognisably thorny issue could await the still-distant technological develop-

ments that would bring Antarctic exploitation closer to the realms of probability. Even seven years later most realistic prognoses tended towards the pessimistic: "... the exploitation of significant deposits", said a leading Australian expert, "presents enormous difficulties. It is very doubtful if minerals could be mined economically in the Antarctic, at least in the foreseeable future."

Plausible though they may have been at the time, such assessments reckoned without the economic downturn of the early 1970s, and when the signatories met in Oslo last June for the Eighth Consultative Meeting under the treaty, Antarctic resources and the likely effects of mineral exploration held centre-stage. The talks were predictably inconclusive, with a standstill agreed pending a full investigation of the environmental hazards; but the expression of environmental concern, though laudable, offers only the thinnest of disguises for the political rationale behind the delay. Exploration surveys, under the guise of scientific research, are well within the bounds of the treaty, but relationships stand to be irreparably undermined if exploitable resources are uncovered and raise again the intractable issue of sovereignty.

Antarctica's resources

The main resources are fossil fuels. Pre-continental drift reconstructions of the former Gondwana continent indicate that most of the Antarctic continental shelf includes thick sedimentary sequences likely to contain fuel reserves. The research vessel *Glomar Challenger* has already found evidence of natural gas beneath the Ross Sea, and an American estimate has put reserves in the eastern shelf at around 115 million million cubic feet of natural gas and 45 thousand million barrels of oil—almost ten times the estimated combined oil reserves of the Brent, Ninian and Forties fields of the North Sea. The chances of uncovering significant deposits have become so good that at the close of the Oslo talks one American delegate was moved to ponder openly the political effect of a rich

strike beneath the zone disputed by Argentina, Britain and Chile.

Evidently the Soviet Union finds the notion equally as intriguing, for less than three weeks later it announced plans to open a new survey base on the Filchner Ice Shelf at the southern end of the Weddell Sea, an area within the disputed territory. Though in itself not a contravention of the treaty, the development is significant because the Soviet Union has never previously shown any interest whatsoever in that part of Antarctica. The Soviet targets are clearly fossil fuels and metallic ores, and it does not require an unduly cynical appraisal to conclude that the main objective of the move is to force an early definition of mineral exploitation rights. "The eastern part of the region", the official statement said, "has many common features with South Africa, the south is reminiscent of the ore-bearing zones of Siberia, while the west is a continuation of the American mountains, famous for their deposits of non-ferrous minerals."

The statement in no way overstates the possibilities, and even though prospecting techniques will be hampered by the enormous overlay of ice, the chances of small Soviet successes must be rated as reasonable. The sedimentary accumulation beneath the Weddell Sea, like those beneath the Ross and Bellingshausen seas, includes up to 15,000 feet of potentially productive strata. The once-contiguous continental shelves off South Africa and South America are already known to contain small fossil fuel reserves.

Opportunities for the discovery of coal reserves and mineral deposits are equally as good on the main landmass. The extensive coal deposits which crop out throughout eastern Antarctica, particularly in the Horlick, Prince Charles and Pensacola Mountains, and in Victoria Land and the George V Coast, suggest that reserves within Permian sandstones may extend at depth beneath the entire continent. As for metallic and non-metallic minerals, over 200 species have been recorded from localities throughout the continent over the past 75 years. Chromium, cobalt, copper, diamond, manganese, mercury, molybdenum, platinum and uranium are all present, and trace quantities of silver and gold, in amounts of up to 2 and 10 parts per million respectively, have been found in pyrite-rich rocks on the Antarctic Peninsula.

But even after the recent adjustments of world commodity prices, has the exploitation of Antarctic resources become an economic possibility? The answer for the moment must be no. Extracting fossil fuels is hazardous enough in the North Sea, where tens of lives are lost to the industry annually,

and where unpredictable climatic behaviour has already pushed production costs way above the earliest estimates. Conditions in Antarctic waters are considerably worse. The depth of water overlying the Antarctic shelves is more than double the average 700 feet in the North Sea, and the insurmountable obstructions of floating pack ice and icebergs scouring the sea floor mean that even such technological innovations as flexible drilling apparatus are unlikely to prove feasible.

Just as daunting and commercially restrictive are the handicaps on the continent itself. Most deposits are isolated, away from the coast, and cut off by glaciers. Coal beds are in the majority of cases limited in their horizontal extent, and mineral finds are usually reported from glacial moraines, so that original outcrops are unknown. Moreover, quality and tenor are generally poor. As for the other minerals, not even high-value metals such as silver, uranium and gold will become viable failing unlikely upward shifts in world prices. The costs of USA gold production would need approximately to double before Antarctic deposits became economic.

Exceptions

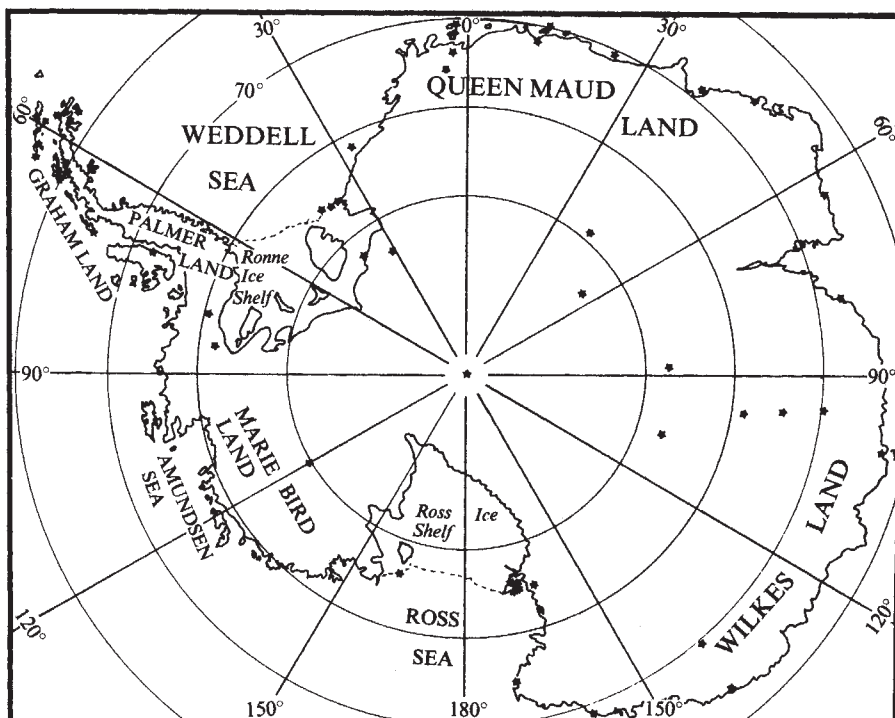
But any thorough assessment of the opportunities must take into account two possible exceptions to this general perspective. The first involves the manganese nodules lining the ocean floor between the continental shelf and the outer boundary of Antarctic territory at the relatively temperate latitude of 60°S. Manganese nodules are present throughout the world, and with

the development of suitable extraction technology are likely to prove an extremely rich resource.

Just as promising is the economic potential of the igneous Dufek Massif in the northern Pensacola Mountains. So far, only viable deposits of iron-bearing magnetite ores have been found, but the complex so closely resembles the richly productive Bushveld intrusion of southern Africa that the prospects have to be viewed in a sanguine light. Further, there is no reason why the Dufek Massif need be unique in Antarctica.

In the final analysis, however, it is the biological resources of Antarctica which currently offer the greatest prospects for this future. Seals are now adequately protected under the Antarctic Treaty, and the whaling industry, which has declined from its level of 15 years ago, will probably continue to do so under the likely build-up of adverse world pressure. Even so, Antarctic waters are stocked with a superabundance of krill, *Euphausia superba*, the small shrimp-like creature forming the staple diet of the whales that visit the region to gorge themselves each year. One estimate has suggested that factory ships could economically take an annual minimum of 270 million tons—an amount far in excess of present yearly world fish catches—without upsetting natural balances.

As for the question of mineral exploitation, this will be discussed when the signatories to the Antarctic Treaty gather in London this summer for a preparatory session ahead of their Ninth Consultative Meeting in Paris next year. □



Antarctica, showing scientific stations in operation 1954-1975

USA

An erosion of eminence?

Colin Norman reports from Washington on a document that examines the strengths and weaknesses in American science

AMERICAN pre-eminence in most areas of science and technology is gradually being eroded, largely because inflation has been eating into funds for research and development in the United States and many other countries have been ploughing large resources into their own science programmes for the past few years. That conclusion emerges from a detailed statistical portrait of American science from 1960 to 1974, published last month by the National Science Board, a committee of eminent scientists which directs the policies of the National Science Foundation.

A cautiously worded document which studiously avoids drawing many conclusions, it attempts to pinpoint strengths and weaknesses in American science and to analyse long-term trends. It covers a vast range of data, including funding levels for research and development, employment statistics, rates of innovation, output of scientific literature and the average number of citations per research paper.

Taken on its own, any one of those indicators probably doesn't mean very much, but together they seem to show a consistent trend—the quality and quantity of American science in relation to that of other countries appears to be headed downwards. The trend, in fact, is so consistent that early drafts of the report are said to have played an important role in securing a large increase for science and technology in the budget announced by President Ford last January. That budget would increase support for research and development by about 11%, while total federal expenditures would only grow by about 5.5%.

Shrinking funds

The clearest and most striking trend brought out in the report is that government funds for research and development have been shrinking steadily in terms of purchasing power since 1968. Support for basic research dwindled by 13% between 1968 and 1974, while expenditures per scientist dropped by a staggering 30% over the same period.

In an attempt to analyse trends in the United States in relation to those in other countries, the report brings out the fact that only two countries—

the United States and France—have shown a long-term decline in the proportion of gross national product spent on science and technology, and the United States is alone in showing a steady decline in the number of scientists and engineers employed in research and development.

Though there have been some significant changes in the pattern of government support for research and development, the report shows that the United States devotes a larger share of its research budget to defence (about 50%) than other countries do. Over the past seven years, however, the share devoted to space science and technology has dwindled, while that devoted to other areas of science has increased.

It is difficult to measure the effect of shrinking support on the overall quality and productivity of American science, but several indicators suggest that the cuts are beginning to show some impact. One measure suggests that the proportion of research papers in several fields of science produced by Americans has reached a plateau; in some cases it has declined. The fields chosen are chemistry, physics, engineering, psychology, molecular biology, systematic biology, and mathematics; in all except chemistry and mathematics, the US still produces the largest share of the world's literature, however.

UK ranks high

As for the quality of research, the report indicates that in every field, research papers produced in the United States are cited on average more often than those produced elsewhere. According to that criterion, papers produced in the United Kingdom rank second in the fields of clinical medicine, biology, and earth and space science, while the UK ranks third in chemistry, physics and engineering. The report does not attempt to analyse whether there are any long-term changes in ranking.

As for technological invention and innovation, some indicators again suggest that American pre-eminence is being eroded by gains in other countries. One measure, the so-called 'patent balance', which relates the number of patents granted to nationals with those granted to foreigners in each country, has been dropping steadily in the United States since 1966. Over the period 1966 to 1973, the number of foreign patents granted in the United States increased by 65%, reaching 30% of the total granted in

1973. That, the report notes, "suggests that the number of patentable ideas of international merit has been growing at a greater rate in other countries than the United States".

Another striking example of that trend shows up in an analysis of rates of innovation. According to a study of some 500 innovations in various areas of science and technology, the US was producing about 80% of the world's innovations in the mid 1950s. By the mid-1960s, the US share had shrunk to about 50%, and although there has been a slight increase since, it has been caused more by a decline in the UK's share than by an improvement in the US position. (The share of technological innovations produced in Britain declined from 25% in 1965 to about 15% in 1973.)

The only indicator which seems to buck the trend of declining US leadership in science and technology is a measure of the balance of trade in high technology products. From 1960 to 1970, the US balance of payments in such products climbed steadily from about \$6,000 million to about \$12,000 million, but between 1972 and 1974, it suddenly jumped to \$25,000 million a year.

Basic research affected

The area most severely affected by declining support in the United States is basic research. In terms of constant dollars, federal support for such research shrunk by some 13% between 1968 and 1974. Physical sciences were the hardest hit, declining by 25%, chiefly because of reductions in the space programme. Those declines have had considerable impact in the universities.

The report notes, for example, that the universities went through a period of rapid expansion until the early 1970s, with faculty numbers in science departments increasing by more than 60% between 1965 and 1972. But, because of declining funds for research, the academic job market has become relatively depressed recently. The result is that the proportion of faculty with tenure has increased from 47 to 65%, and the proportion of young doctoral faculty in postgraduate science and engineering departments declined from 42% in 1968 to 28% in 1974. The median age of faculty members consequently increased from 41 to 44.

Many of the trends and conclusions brought out in the report are hedged with caveats and cautions. The National Science Board hopes to refine some of the measures, and produce an updated report in a couple of years time, however. □

BRITAIN

Changing nuclear fortunes

News last week transformed uncertainty surrounding the reprocessing of nuclear fuel in Britain into certainty. For the Dragon reactor, the reverse seemed to occur. Allan Piper and Roger Woodham report

AFTER several months' delay British Nuclear Fuels Ltd (BNFL) has obtained Government approval for plans to import and reprocess 6,000 tonnes of foreign nuclear fuel. The go-ahead clears the way for an early settlement of the contract for BNFL to handle up to 4,000 tonnes of material from Japan's new generation of light water reactors at its Windscale plant in Cumbria. But further delays are expected following French moves to capture part of the deal, which is worth as much as £600 millions over the decade following 1979.

Mr Con Allday, BNFL's managing director, has indicated that the head of the French reprocessing organisation Compagnie Generale des Matieres Nucleaires (CGMN) may accompany him to Japan later this month. The French intervention, made possible by their involvement in a tripartite reprocessing alliance with Britain and West Germany, could mean the loss to BNFL of up to half of the Japanese contract.

Final settlement of the Japanese deal, which has aroused some controversy in the UK, has been delayed by a Government investigation into the issue. Granting Government approval last week, the Energy Secretary, Mr Anthony Wedgwood Benn, said that

the Government had "given full consideration to the safety and environmental implications of accepting more work of this kind, taking account particularly of the views which have been expressed in the recent extensive public discussion of the question".

It is now clear that all waste, whether reprocessed or not, will eventually return to Japan, and that the deal does not contravene the Non-Proliferation Treaty. In further contracts clients must accept a break clause freeing BNFL if residual waste cannot be treated for safe return in a glassified form.

BNFL have already expressed confidence that successful completion of the Japanese negotiations will bring in other orders, and Iran, Italy, Spain, Sweden and West Germany have been unofficially mentioned in this connection. To meet projections for oxide reprocessing by 1990 BNFL plan to invest £900 millions in two new reprocessing plants at Windscale.

Meanwhile, the moribund Dragon High Temperature Reactor Project continues to attract attention. Whatever the politicians in Brussels may decide about a data retrieval programme to get the most out of the Dragon project, a sum of about £700,000 has already been committed by other interested parties to this end. The German nuclear research centre, KFA Jülich, has chipped in £480,000 to make sure that all the information it needs for its work on high temperature reactors is wrung out of the project, and even the European Commission and the United States Energy Research and Development Administration

(which eventually declined at the end of last year to help keep the project afloat) are contributing to a brief continuation of the programme concerning the effects of helium in the primary cooling circuit on the metals used there. This work is likely to continue to the end of the year and involves carrying on existing Dragon contracts with the Norwegian research centre CIIR and the Fiat company in Turin.

Apart from the £130,000 which it is making available for this venture, KFA Jülich is also providing £350,000 towards a seemingly winding-up of the work at Dragon on fuel elements, in which it has a particular interest. This will involve what remains of the Dragon staff in post-irradiation experiments on the test fuel, some of which is still in the reactor.

The German research centre does, however, stand to recoup that £350,000 if a more comprehensive plan put up by the European Commission survives the Brussels obstacle course. The commission is suggesting a data retrieval programme costing £1.16 million, of which it would pick up the tab for 90%—the assumption being that some of the smaller countries that have participated in Dragon would stump up the remainder, and that the UK Atomic Energy Authority would contribute nothing.

The indications are, however, that the polarisation of political views in the Council of Ministers is such that even this—an expenditure of some 2% of the total amount of money spent on Dragon since its inception in 1959 to see the project decently buried—is by no means certain to be acceptable. And, in addition, some of the smaller countries associated with the project have simply given up in disgust. □

AUSTRALIA

Probing the conservative mind

Peter Pockley reports from Sydney on the latest developments affecting Australia's scientific community three months into the new administration

SHELTERED from the blaze of publicity surrounding the activities of the Federal politicians as they jockeyed for position before the opening of the new parliament, Australian scientists have been working behind the scenes to protect their interests. During January and February, the Prime Minister, Mr Malcolm Fraser, was busy putting the axe into a number of Labor-initiated inquiries, commissions and authorities.

As the Canberra grapevine identified the descending blades, all manner of interested groups hurriedly sought ways of protecting their areas of interest and influence. Some lost their heads in the scramble, partly for want of comprehending the conservative mind now in power. The leaders of the scientific community, though, were by then well versed in political techniques, and their burst of frenetic activity had considerable, possibly long-lasting, effect.

ASTEC survives—just

In the eyes of the scientific lobbyists, the principal institution to defend was the fledgling Interim Australian Science and Technology Council



Chairing ASTEC review: Dr Louis Matheson

(ASTEC), which was under very real threat of abolition or absorption within the public service structure. To the

surprise of some, for as Prime Minister he was largely an unknown quantity, Mr Fraser proved accessible to the principal lobbyists, and he demonstrated a close, personal interest in science matters.

In the few months of its existence under Labor, ASTEC had begun to work well, but this could only be perceived on an internal basis. It had not had time to show results in the form of well-considered reports and advice to government. The dozen members had defined the major policy targets to examine in depth, and they set about doing so through a series of "Task Forces", each comprising members of ASTEC and co-opted outside experts.

Task Forces on Energy, Health Science, Social Science, Marine Science and Public Understanding of Science were established as going concerns by the time Mr Whitlam's government was defeated at the polls in December. Further Task Forces on Industrial Research and National Science Budget were formed but had not met. Some criticism had been levelled at ASTEC for allowing itself to take on too much in the way of tactical detail. Some of this detail was no fault of the members, though, since quick advice was sought on occasions by the then Labor Minister for Science and Consumer Affairs, Mr Clyde Cameron.

A broader strategic approach was favoured by these critics, but it is likely

that this would have evolved naturally anyway. The existence of the criticism, however, assisted the anti-ASTEC arguments of the Department of Science following the election. Butterflies fluttered in the bellies of those scientists who saw in ASTEC their best, and only, hope for policy advice which is independent of the large government establishments dominating the Australian science scene.

At the end of January, Mr Fraser published his list of cuts and abbreviations in the inquiry industry. Although many of the cuts were largely cosmetic, they carried the useful public relations impression of a tough hand at the helm of economic management by first curtailing public expenditure. It was nonetheless important that ASTEC did not appear on the list. For those involved, a further fortnight of anxiety followed, caused by the continuing uncertainties of learning to deal with a new political regime. Then, Mr Fraser personally announced the formation of an Advisory Group to advise him—significantly him—"on the role of a continuing Science and Technology Council, its terms of reference and all other matters concerning its operation."

Dr Louis Matheson, the part-time Chairman appointed by Labor to ASTEC with expectation of full-time status, is chairing the review, again in a part-time capacity. Three of the four other members are "heavies" of the Academy of Science; the numbers are

made up by a retired industrialist.

The very existence of the review, now working actively, and the nature of its membership point to a recommendation for the continuance of ASTEC, but whether, in the context of a public service-oriented government, the Labor ideal of statutory independence can be sustained is more doubtful.

Labor disarray

The traumatic period of adjustment to the status of an Opposition is still eating into the heart of the Labor Party, and it is not surprising that they have been silent on science policy matters. They seem to have been too busy tearing each other apart (notably trying to downgrade their re-elected leader, Mr Gough Whitlam) to probe the government's uncertainties on science policy. The Labor shadow cabinet is an elected body from the caucus of members of both Houses. Mr. Whitlam was saddled with some shadow ministers whom he would rather not have seen again, such as Mr Rex Connor, whom he had sacked as Minister for Minerals and Energy when in government, and Mr Clyde Cameron, whom he had demoted to the portfolio of Science and Consumer Affairs.

Mr Whitlam's one area of choice were the "portfolios" allocated to his front bench. Mr Connor became spokesman for science. So far, the shadow has not materialised into substance. □

COMECON

THE Interkosmos programme for joint Comecon research in space has just celebrated its tenth anniversary. The intention behind the programme was to carry out research into four major fields—space physics, meteorology, communications and space biology and medicine. According to its chairman, Academician B. N. Petrov, it was envisaged as a co-operative effort by all Comecon countries, whose scientists "jointly drew up a catalogue" of the most interesting problems in space research. The Soviet Union has, of course, always been responsible for providing the rockets and launch facilities to place the Interkosmos probes in orbit, and theoretically all the member countries should provide instrumentation. In fact, the Soviet participants provide the major share; out of five experiments in the recent Interkosmos-14, three were of Soviet provenance, one came from Czechoslovakia and one from Bulgaria. This imbalance is despite the fact that some member states are competent to provide experiments—Poland, Czechoslovakia, Rumania and Hungary were repre-

sented, as well as France and the USA, in the experiments carried by the biological satellite Kosmos-782.

● Aid to Mongolia from the developed members of Comecon was an issue discussed when the Comecon Committee on Scientific and Technical Cooperation met at Budapest in January. Mongolia joined Comecon in 1962, and is now seen as a potential source of mineral wealth: teams from the Soviet Union, Hungary, the GDR, Poland and Czechoslovakia have carried out exhaustive geological surveys during recent years, making important finds of coal, copper, tin, gold, phosphorites and kaolin; prospecting is also going forward for tungsten, fluorite and zinc. Over the last three years, the Standing Commission of Comecon has been working out an integrated economic plan for the development of these resources. In addition to assisting with these surveying projects, the European members of Comecon will, during the coming Five Year Plan, assist the development of Mongolian science and technology in a number of ways, including the establishment

of laboratories for genetics, radio-electronics, and the analysis of fuels and lubricants.

● Cooperation in the agricultural sciences between the USSR and the other member countries is an important aspect of Comecon policy, and a recent agreement between Hungary and the Soviet Union envisages joint research into the procurement, storage and processing of cereals and the production of mixed fodder. Agricultural experts in the two countries, says the Soviet Minister of Procurement, are working on a number of identical problems. But one particular problem of Hungarian agriculture—the sugar beet harvest—seems to have arisen directly as a result of copying Soviet models.

Hungarian agriculture has embarked on an extensive programme of "chemicisation", and, according to Budapest radio, while the 1975 campaign to raise sugar beet production increased both the area under beet and the crop-density, the sugar yield of the beet was actually lower than the pre-campaign level.

Vera Rich

IN BRIEF

EEC at sea

Member states of the EEC, which is currently preparing itself for 200-mile community fishing limits, went to the fourth session of the Law of the Sea Conference this week still facing a problem over national limits. Responding to the resistance expressed to the idea of a 12-mile limit by James Callaghan, the UK Foreign Secretary, at the Foreign Ministers meeting a fortnight ago, the EEC Agriculture Commissioner, M Pierre Lardinois, said last week that any extension of exclusive national limits beyond 12 miles would require a reversal of the Treaty of Accession, and indicated that catch quotas might offer a means of achieving the desired end. Delegates from over 150 countries are in New York for the Law of the Sea Conference, and apart from the problem of coastal states' jurisdiction, they are expected to tackle the issue of exclusive economic zones, particularly in relation to fishing and deep-sea mining.

ABRC confirms cutbacks

Figures contained in appendices to the

second report (published this week) of the UK Advisory Board for the Research Councils (ABRC), which advises the Minister of Education and Science on the allocation of the science budget amongst the research councils, confirms the downward trend indicated by the recent public expenditure White Paper. The distribution of the £216 million science budget for 1976-77 shows that, while overall growth in real terms will amount to 1.7%, the Science Research Council (SRC) will experience a 1.6% decline. The SRC's share of the total will fall from 54.2% now to about 52% in 1980-81. The proportion going to "big science" will fall even faster, from 34% to 27%.

Asbestos check

The UK Health and Safety Executive is reportedly investigating compliance with and enforcement of the precautions contained in the 1969 Asbestos Regulations. The immediate cause of concern is a suspected link between mesothelioma (a malignant tumour in the stomach or chest) and exposure to asbestos.

Kissinger on nuclear trade

Speaking before a Senate committee last week, the US Secretary of State, Dr Henry Kissinger, stated his opposition to specific proposals designed to tighten control of international trade in nuclear technology, and rejected suggestions that the USA and USSR should jointly urge countries like France and West Germany not to export nuclear facilities. The proposals, Dr Kissinger said, would violate the spirit of international undertakings by the US.

Uranium resources report

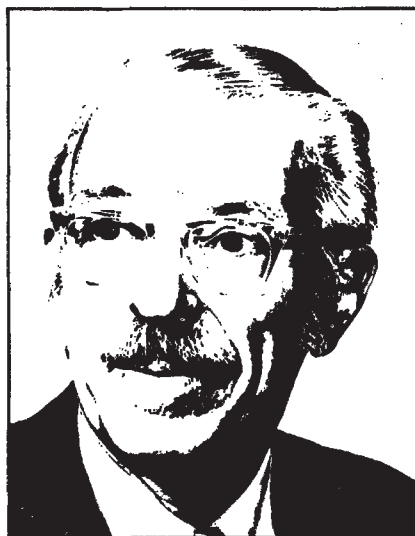
A report on uranium resources produced by the OECD's Nuclear Energy Agency (NEA) and the International Atomic Energy Agency (IAEA) estimates that demand for natural uranium will increase from the present 18,000 tonnes a year to about 50,000 tonnes by 1980 and possibly 100,000 tonnes by 1985. Demand up to the next decade, the report indicates, could be met, but thereafter much will also depend on recycling and prevailing prices as well as new discoveries.

ONE place in California exceeds all the rest in scenery and fame: Yosemite Valley, a national and international shrine of acclaimed splendour. Two Congressional subcommittees have just recommended that the US Interior Department take immediate action to prevent Yosemite from becoming "a major Walt Disney-type attraction". Hopefully, the analogy bodes ill for an actual plan by Disney to destroy another mountain treasure: Mineral King in the southern Sierra Nevada, where restaurants, chair-lifts and garages have been proposed.

I have not seen the report, but I know some of the circumstances leading to it such as the egregious and repulsive proposal that a tramway be built on Glacier Point. This wretched idea was turned down, but the very suggestion should have been enough to call for immediate expulsion of its proponent, the Music Corporation of America (MCA), from the concessions now assigned to MCA in Yosemite.

The Valley has been described in the prose of John Muir and the poetic photography of Ansel Adams. I can do no more than footnote their achievements. Yosemite, the goddess of fatal beauty, in spring is the deep roar of stupendous waterfalls, profusions of azaleas and dogwood blossoms, soaring sculptures in immense

granite cliffs, the fragrance of ponderosa pine trees mingled with new foliage of black oaks and green leaves of manzanita, the harsh call of dark-crested Steller jays.

Ravished goddess

THOMAS H. JUKES

A rock-climber, Yvon Chouinard, has spoken of, in summer

"the tremendous heat, the dirt-filled cracks, . . . the filth and noise of Camp 4 (the climbers' campground), and, worst of all, the multitudes of tourists . . . but then there are times when I should rather be here than anywhere else in

the world . . . It is a strange, passionate love that I feel for this Valley"

Clinging to the sun-warmed rock, high above the valley floor, a climber sees Yosemite better than anyone else. The sounds below fade to a faint murmur.

Golf courses, tennis courts, a bank, service stations and garages, barber and beauty shops, and nineteen places where liquor can be purchased, have invaded the delicate tissues of Yosemite like a festering sore. The best therapy would be debridement. All buildings could be removed by using the equipment and machinery that work so successfully for this purpose in run-down urban areas. It would, of course, defeat the basis of the restoration if visitors were kept out of the Valley, but the less agile could travel in sight-seeing buses (many do so now) and the more able-bodied could move on bicycles or on foot, thus combining the benefits of scenery and exercise. (Last October, I gave a lecture on nutrition in an auditorium in Yosemite. I should have asked the audience, in the name of John Muir, to leave the building, forget the meeting, and walk with me to Vernal Falls).

These changes, which are doubtless too radical to be considered, would disrupt jobs and sensibilities. But their adoption would do nothing but good to Yosemite Valley.

correspondence

Triggered lightning hazards

SIR,—Following the publication of our paper entitled "Artificially triggered lightning above land" (*Nature*, **257**, 212; 1975) we hear that several specialists elsewhere are thinking of embarking on similar programmes. Therefore, safety considerations prompt us to discuss some recent findings about unexpected lightning strokes to the ground in the vicinity of such experiments.

Among a total of 45 successful triggerings using a rocket carrying a thin steel wire attached to the ground, the following sequence of events occurred on six occasions. First, when the ascending rocket was more than 150 m high, the device attracted a small current detected only because the vaporised wire emitted a faint glow. This glow passed through a maximum and then began to decrease. Between 40 and 80 milliseconds later, a bright flash occurred striking the ground or an object in the near neighbourhood. The maximum distance between the expected and actual point at which the lightning struck has been as much as 145 m.

Evidence obtained at various distances with still and movie cameras reveals that in the upper region the arrangement of the luminous branches is typical of upward development, whereas in the lower part the streamer shapes indicate downward propagation. Therefore it is likely that the main channel is created by propagation in both directions, starting from the upper portion of the wire.

The photograph is of an anomalously triggered flash striking the ground near our experimental site. Another flash which struck a pine grove damaged several trees and in the next twelve months these and adjacent ones died.



The anomalous triggering effect is of great interest but calls for more stringent safety measures in the experimental area. A detailed report is available on request.

Yours faithfully,

R. FIEUX

*Electricité de France,
Division des Etudes et Recherches,
F-92 140 Clamart*

P. HUBERT

*Commissariat à l'Energie Atomique,
F-91 190 Saclay, France*

CPSU Congress

SIR—A notable feature of the recent Twenty-Fifth Congress of the Soviet Communist Party is the adherence of the hard-core communists to Karl Marx's principle of the scientific interpretation of history.

Mr Pytor Masherov, party chief of the Byelorussian Republic, in an attack on some Western communists, said: "To say Marxism-Leninism has become antiquated is like saying that Newton's 300-year-old law of gravity is antiquated too".

Mr Gus Hall, the US Communist Party Chief, went a step further. He said: "The ideas of Marxism-Leninism penetrate the whole contents of Leonid Brezhnev's speech like the bright ray of a powerful laser".

Perhaps in the following congress we shall hear of quasars, black-holes, big bangs, etc. As a scientific community we should feel elated!

Yours faithfully,

SREEDHAN ROY

*Department of Mechanical
Engineering,
University College,
London, UK*

The pace of life

SIR,—Bornstein and Bornstein's paper (February 19, page 557) leads to fascinating conclusions. Their data suggest that in the case of the smallest human conurbation (one person) the average walking speed is 0.05 feet s^{-1} (50 m h^{-1} or 1.3 km d^{-1}). Fortunately this quantity is not negative. On the other hand, its value is small and a value of zero is probably compatible with the experimental data. This may be the explanation of the fact that those few people who are known to live alone show a propensity for spending extended periods of time on the tops of poles, in caves or in barrels.

Yours faithfully,

J. A. EADES

*H. H. Wills Physics Laboratory,
Bristol University,
Bristol 8, UK*



Competition 6

LITTLE jingles are being put in advertisements by the British Metrication Board to speed the public's passage into a metrical world. Specimen—

Two and a quarter pounds of jam,
Weigh about a kilogram.

A prize of £10 is offered for the best rhyming couplet on one of those forgettable SI units. Closing date for entries is April 21.

Competition 5 demanded an anagram on a scientific concept to rival the irreverent one on *Nessiteras rhombopteryx*. There was a large entry—most people couldn't stop when they got into the swing of things.

To W. A. F. Watson of Aberdeen

goes £6 for "Fie, Mendel! Result cheating, score *all* peas!" (These DNA molecules are self replicating).

H. J. Taylor of Sheffield wins £4 for "Natural Science men musn't interpolate inapt ideas" (*Entia non sunt multiplicanda praeter necessitatem*).

C. K. Thornhill of Orpington received honorable mention for "O, so in fact G not t-variant?" (Constant of gravitation). Hard luck on several good candidates whose anagrams weren't.

news and views

First mammalian results with genetic recombinants

from Bob Williamson

BREAKTHROUGHS in science occur in weird and wonderful ways. The scenarios for genetic engineering (forming recombinant molecules of bacterial virus DNA and animal or plant DNA) vary, as most now know, through the entire gamut of the fanciful—from joy to horror, depending more upon the mood of the speaker and the receptiveness of the audience than on anything as mundane as fact. During the past 2 months four reports have appeared of the first integration of portions of a mammalian gene into a bacterial plasmid—as might be expected, a portion of the gene coding for the protein haemoglobin. In the event, the recombinants obtained well illustrate the tremendous technical usefulness of genetic engineering, with little of the fiction one way or the other. For the next few years at least, recombinants will provide molecular biologists with a versatile tool to prepare pure probes with which to dissect the fine structures of genes and the way they are expressed. The experiments which will be done have been attempted before, but the probes will now be better.

Basically, all four groups have made a double-strand copy in DNA of purified globin messenger RNA prepared from rabbit reticulocytes. Since the mRNA is already very pure (as judged by direct sequence analysis, among other things) there is little chance of introducing any Pandora's box of nasties into the plasmid—the experiment must be judged one of the safest imaginable, much safer than any planned integration of DNA fractions made from mammalian cell nuclei.

In each case the first step is to make a single-strand copy of the globin mRNA with DNA-dependent RNA polymerase, the 'reverse transcriptase' isolated from tumour viruses. Rougeon, Kourilsky and Mach (*Nucleic Acids Res.*, **2**, 2365; 1975) from Geneva and Paris, prepared this in the conventional way, and then made the complementary DNA strand using DNA polymerase, giving a double-strand DNA indistinguishable from part of the globin gene. (Since cDNA is rarely a full length copy of mRNA, it is likely that the double strand globin gene DNA is only partial

as well, though full data on this is not yet available.) This 'gene' was then elongated with another enzyme, terminal transferase, which adds homopolymer tails, in this case of oligo(dG), to the 3'-ends of both strands of DNA.

A plasmid, pCRI, was then joined to the partial copy of the double-strand globin gene. pCRI is derived from colEI-kan, which can be selected because of the resistance to the antibiotic kanamycin it confers on infected cells. It grows in that favourite organism of bacterial geneticists, *Escherichia coli*. The plasmid used is, like all plasmids, circular, but can be broken in just one specific place by the bacterial restriction nuclease *EcoRI*. This linear plasmid DNA was first repaired with reverse transcriptase to reconstruct the double strands at the overlapping cuts of the *EcoRI* enzyme, and then oligo(dC) was added to both 3'-ends (again with terminal transferase).

The plasmid DNA, with its oligo(dC) tails, was mixed with the globin gene, with its oligo(dG) tails—strong hydrogen bonds form between oligo(dC) and oligo(dG). The globin gene with tails 'fills in' the rest of the nicked circle—only plasmids picking up globin genes are circular, and only a circular plasmid is infective. Therefore only recombinants which have picked up the added globin genes will grow and multiply in the bacteria. When it enters an *E. coli* cell the plasmid is held together only by hydrogen bonds, but the bacterium does the rest, and repairs the plasmid by filling in any gaps and forming covalent closed circles. The elegance of Rougeon's experimental approach is that the inserted fragment can in theory be cut out again using restriction enzymes, since each R1-sensitive site has been carefully reconstructed on either side of the inserted gene.

The results are not quite so simple—the efficiency of transformation is still rather low, and most recombinants do not contain inserted fragments—but at least one of the plasmids isolated contains a portion of the rabbit β -globin gene, as shown by hybridisation to globin cDNA (single stranded, this time) enriched in β -globin sequences. The grapevine (extraordinarily efficient

in this field—I find it hard to imagine anyone getting a positive result without everyone else knowing within days at most) tells us that an α -globin gene recombinant has also been prepared by this group.

Tom Maniatis and his colleagues at Cold Spring Harbor and Harvard (Efstratiadis Kafatos, Maxam and Maniatis, *Cell*, February, 1976) have chosen a somewhat simpler technique to make full-length double-strand cDNA once again using rabbit globin mRNA as the starting material. Single-strand cDNA is first prepared with reverse transcriptase, and this acts as a template for a second strand made with DNA polymerase, even in the absence of added primer. The double-strand cDNA was shown to be a single polynucleotide chain, looped round at the end, the primer being part of the original single-strand cDNA. The loop can be cut with single-strand specific S1-nuclease and each of the strands elongated with terminal transferase. Plasmid recombinants containing both α - and β -globin gene sequences were obtained. Full experimental details of the recombination and transformation are not given and will follow in a later paper, but it is likely that a poly(dA)–poly(dT) hybrid was used to link the cleaved plasmid and the globin gene fragment. Higuchi, Paddock and Salser, of the University of California, Los Angeles, have independently carried out similar experiments linking rabbit globin double-strand cDNA to plasmid pSC101 by poly(dA)–poly(dT) tails and have obtained at least 28 recombinants. They have been able to demonstrate in some cases that the inserted DNA truly codes for the globin sequence by the presence in this sequence of a specific R1 restriction endonuclease site shown to be present by this group's previous RNA sequence analysis.

Several British laboratories have also been interested in this technique, and the first person to report a positive result is Terry Rabbitts, of the MRC Molecular Biology Laboratory at Cambridge (this issue of *Nature*, page 221). He has used a different and elegant trick to integrate the gene. First, a related *E. coli* plasmid (mini-ColEI) was cut with restriction enzyme to give

a linear molecule as before, and then oligo(dT) added directly to the cut ends. Rabbit globin mRNA binds to this oligo(dT) by its poly(A) tail, and the cDNA was synthesised with reverse transcriptase directly onto the plasmid. Poly(dT) tails were again added, this time to the globin cDNA. The mini-ColEIs containing globin cDNA were melted and reannealed with plasmids containing no globin cDNA but with a poly(dA) tail. Circles form between elongated cDNA-containing plasmids (containing poly(dT)) and strands from those with poly(dA) and when these infect *E. coli*, once again the bacterium repairs them, seals them up and the plasmids replicate normally.

Maniatis, Rabbitts and Salser all finish with a piece of rabbit globin gene bound covalently to plasmid by two oligo(dA)-oligo(dT) stretches, which cannot be cut by an available enzyme as neatly and easily as Rougeon's R1-sites. dA-dT hybrids are less stable than normal DNA double strands, and when the temperature goes up or the salt concentration goes down, they melt first, and form little single-strand bubbles while both inserted globin gene and original bacterial plasmid remain annealed. Therefore they can be broken by nucleases (such as S1) that attack single-stranded DNA preferentially. While not as elegant as Rougeon's technique for removing the inserted DNA, this should prove quite as effective.

One worry of bacterial geneticists has been stability of these recombinants—will they keep growing in culture, or will the inserted gene be eliminated? The two sequences (whether oligo(dA)-

oligo(dT), or R1 sites) at either side of the insert could provide an active cross-over site for recombination with elimination of the mammalian DNA. Since the newly made recombinant grows more slowly than the original plasmid, any revertants would outgrow the carefully made hybrid plasmids. Fortunately, this does not seem to happen at a very fast rate, since the hybrids are stable for several months at least.

Ironically, only one of the four labs reporting so far is particularly interested in globin genes—several groups have already shown that α -globin or β -globin-specific cDNA can be prepared using other techniques, albeit rather tediously. Salser and his colleagues, however, should find the new technique very useful in their attempts to sequence globin mRNA. Both Rougeon and Rabbitts have been hoping to purify sequences complementary to mouse immunoglobulin mRNAs; Maniatis' colleagues hope to study the appearance of a complex series of mRNAs during insect development. The problem for all these groups in extending this technique is the purity of their mRNAs. In the case of immunoglobulin, it has proved very difficult to get mRNA greater than about 60% pure; the insect mRNAs are an even more complex mixture. Using genetic recombinants, scientists could now clone pure populations of double-strand cDNAs derived from each mRNA separately. Separating α - and β -globin genes represents a simple model system to this end. The fact that this has proved so simple will be very encouraging to people studying more

complex systems.

The recent National Institutes of Health guidelines (outlined in part in *Nature*, 258, 561; 1975) suggested that the containment facilities used should be mentioned by groups publishing in this field, to act as a guide to others. It is appropriate that one of these papers includes such a discussion—particularly as the guidelines are only just available. No doubt the full reports from the other groups, when published, will follow suit. The fact that the recommended safe procedures are followed for completely non-hazardous experiments should help to ensure that they are followed in the more delicate experiments which will no doubt be reported soon.

One last point—at the 'big' Asilomar meeting a year ago, several of us suggested that anyone obtaining a recombinant such as those discussed here should make it available to other laboratories as soon as the data on it have been published. This is because the major 'danger'—in this case virtually nil, but in future cases perhaps more substantial—arises during the first recombination and isolation of the plasmid containing the foreign genes, when the DNA integrated is not yet fully characterised. The power of these techniques is such that many others will be tempted to follow on immediately, perhaps with less experience and poorer facilities. I only hope that Rougeon, Rabbitts, Maniatis, Salser and their co-workers and followers will make their recombinants freely available. In this field, the way we start will determine whether others will act responsibly later. □

Great step forward for multiple sclerosis research

from Cedric Mims

RESEARCH into multiple sclerosis (MS) has been in a somewhat rejuvenated, exciting state ever since the discovery 10 years ago that two uncommon diseases of the central nervous system of man, subacute sclerosing panencephalitis and progressive multifocal leucoencephalopathy were both caused by viruses (measles and a new human papovavirus). At about the same time two other rare neurological diseases, Kuru and Creutzfeldt-Jacob disease were found to be infectious in origin, and transmissible to subhuman primates. The causative agents in this case were unique virus-sized microorganisms, very similar to scrapie, and the incubation period was exceedingly long, occupying a large fraction of a life time. Earlier epidemiological studies had pointed to a possible infectious

origin for MS, and these findings stimulated a serious hunt for a virus.

For some years a favourite candidate for the MS virus has been measles, but the evidence has been indirect, based on the high titres of antibody to measles found in the blood and cerebrospinal fluid of patients with MS. But not all patients have raised antibodies to measles, and antibodies to viruses such as mumps and rubella may also be elevated (Norrbj *et al.*, *Infect. Immun.*, 10, 688; 1974). The association of MS with certain histocompatibility types (HLA 3 and 7) has prompted various virological suggestions. Perhaps the histocompatibility antigens on the cell surface act as virus receptors and thus promote susceptibility to MS, or perhaps certain immune response genes linked to these

histocompatibility genes determine the immune response to a virus responsible for MS. A recent modest sized survey from Canada, however (Lamoureux *et al.*, *Brit. med. J.*, Jan 24, 183; 1976) indicates that patients with MS have unusual antibody responses to a variety of different antigens, antibody levels to certain viruses and to tetanus and diphtheria being lower than normal.

There have been a number of false alarms, virologically speaking, and when Dick Carp and his colleagues from the Institute for Research in Mental Retardation, Staten Island, New York, reported on a transmissible factor present in the brains and serum of MS patients (*J. exp. Med.*, 136, 618; 1972), this was greeted with some scepticism, especially because the transmissible factor could only be demonstrated

by its ability to decrease the number of circulating polymorphonuclear leukocytes in the blood of infected mice. The agent was titrated, and MS brains were found to have about 10^{12} infectious units per gram; it passed 50 but not 25 nm filters, and was serially transmissible in mice. Although it replicated after injection into mice there were no other signs of infection. But the test for a fall in circulating polymorphs was very difficult to standardise. These cells are notoriously variable in number, often difficult to identify in mouse blood films, and other laboratories were unable to confirm the findings. Carp then found that the same transmissible agent when added to a line of cultured mouse fibroblasts, caused a slowing of the rate of cell division, and this effect could be titrated out and the agent serially passaged through the cultures (*Infect. Immun.*, **9**, 1011; 1974). He later extended these findings, and described reduced cell yields of cultures after inoculation with various MS tissues, including brain, spleen, serum and cerebrospinal fluid (Carp *et al.*, *Neurology*, **25**, 492; 1975). As if to further strain our credulity his laboratory had reported that brains from mice infected with scrapie also caused a lowering of circulating polymorphs in mice (*Infect. Immun.*, **6**, 370; 1972). Titres were high and by diluting out his inocula, he was able to separate the polymorph lowering agent from scrapie itself (*J. infect. Dis.*, **128**, 256; 1973). The polymorph lowering effect was confirmed with scrapie from a variety of sources in a publication from Dickinson's laboratory in Edinburgh (*Nature*, **248**, 510; 1974). So far there have been no reports of reduced cell yields in tissue cultures inoculated with the scrapie-associated agent.

The status of all this work was raised dramatically by the recent publication of two papers from the Henles' laboratory in Philadelphia (Koldovsky *et al.*, *Infect. Immun.*, **12**, 1355; 1975; Henle *et al.*, *ibid*, **12**, 1367; 1975). They too found that the brains and serum from MS patients contain an agent that depresses the polymorph counts of mice. The effect was seen not only in all strains of mice tested, but also in rats, hamsters and guinea pigs. Titres were up to 10^9 infectious doses per gram in MS brain, and up to 10^3 in MS serum. The multiple sclerosis-associated agent (MSAA) was transmissible from animal to animal and passed through 50 nm but not 25 nm millipore filters. Perhaps the most important and convincing addition to Carp's work was the demonstration that nearly all MS sera and cerebrospinal fluids contained antibodies that neutralised the polymorph lowering effect. Many of the MS patients' relatives and nursing per-

sonnel also had serum antibodies, whereas only 1 of 59 sera from people without MS contacts had antibodies. Interestingly, 37 of 68 sera from East Africa were positive.

The evidence, therefore, is for a new microorganism infecting man, somewhere between poliomyelitis and polyoma virus in size, of unknown structure and nucleic acid, and strikingly associated with multiple sclerosis. Although the agent is present in large amounts in MS brains, and should be 25 to 50 nm in diameter, it has so far not been seen in the electron microscope. The long and mostly fruitless searches for virus-like bodies in MS brains make it seem unlikely that it will turn out to be a regularly sized or shaped object.

The stage is now set for intensive investigation of this new microorganism. It is especially to be hoped that there will be confirmation of Carp's report that the agent replicates and causes detectable changes in cultured cells. Strictly speaking the MSAA could turn out to be merely associated with some as yet undiscovered agent more directly responsible for MS, in much the same way that the other polymorph-lowering agent (possibly related to the MSAA) could be separated from scrapie. In recent years scrapie and MS have seemed to have been drifting away from each other, but there is much in these recent findings to suggest a closer association. Perhaps the MSAA is also membrane-associated as suggested by the susceptibility of both MSAA and scrapie to periodate. If both of these agents are mere membrane fragments or vesicles and of nondescript structure, then the failure so far to demonstrate either by electron microscopy is more understandable. One important difference, however, is that scrapie gives no sign of inducing the faintest flicker of an immune response in the infected host, whereas specific antibodies seem to be formed to the MSAA. If we accept the work on the scrapie-associated agent, scrapie becomes much more relevant for MS, reminding us that medical virologists neglect the field of veterinary virology at their peril.

Even if multiple sclerosis is an infectious disease, it is still vital to study the "soil" (host) as well as the "seed" (virus). Biochemical, cell culture, immunological and other investigations have been of great importance in MS research and they can now be oriented towards the associated microorganism. There is evidence for a strong immunological component in the disease process, and sophisticated immunological techniques can be brought to bear on this matter now that antigens of a specific microorganism seem to be available.

A hardened investigator might well at this stage still have some doubts and reservations about all this work, but it has given a fresh transfusion of life and hope to MS research. Evidently the new agent poses formidable technical problems, but it will nevertheless be further investigated and characterised, while serological studies should help to provide us with some understanding of its epidemiological behaviour. Finally if the agent proves to be causative rather than merely associated with MS, a vaccine could perhaps be produced to prevent infection and thus prevent the disease. □

Transferring nitrogen fixation genes

from J. E. Beringer

DURING the past few years considerable interest has centred on *in vitro* methods of joining together fragments of DNA from different sources, and the advantages and disadvantages of what is now called 'genetic engineering' have been the subject of much debate. This debate has tended to obscure the fact that some plasmids, such as the *Escherichia coli* sex factor F, have long been known to be able to pick up chromosomal DNA *in vivo* forming F' factors. It is timely, therefore, that two papers in this issue of *Nature* (pages 268, 271) should remind us of the type of genetic manipulation that can be done *in vivo* and also show that such techniques are not only academically interesting, but have a real use—in this case to facilitate genetic studies of nitrogen-fixation genes. These papers by Dixon, Cannon and Kondrosi, and Cannon and Postgate describe first the transfer of *Klebsiella pneumoniae* nitrogen-fixation (Nif) genes from an F', which has a very limited host range, to the plasmid RP4, which has a very wide host range, and then report on the transfer and functioning of these genes in such diverse bacteria as *K. pneumoniae*, *Rhizobium meliloti* and *Azotobacter vinelandii*.

Two particularly interesting points arise from these reports. The first concerns the transfer of this plasmid to *A. vinelandii*. Cannon and Postgate were able to show that the *Klebsiella* Nif genes it carries could overcome mutations in *A. vinelandii* that have resulted in the loss of either component I or component II activity of the enzyme nitrogenase. Whether functional hybrid nitrogenases were formed in *A. vinelandii* remains to be demonstrated. If this is indeed the case, and it also applies for other Gram-negative nitrogen fixers, then genetic studies of the *Klebsiella* Nif genes will not only

provide a model for studies of nitrogen-fixation genes, but may also enable one to produce a range of plasmids carrying mutations in known Nif genes that can be used for complementation studies of these genes in other bacteria. Furthermore, studies of the transfer and expression of the *Klebsiella* Nif genes in a range of nitrogen-fixing and non-fixing bacteria should help to answer some of the simplest questions that can be asked about the feasibility of performing wide intergeneric transfers of nitrogen-fixation genes. In this respect the observation that transfer to *Pseudomonas aeruginosa* did not occur is slightly discouraging, since RP4 (the parent plasmid) was isolated in this organism and a derivative of it carrying the *E. coli* histidine operon produced by Olsen and Gonzalez (*Biochem. biophys. Res. Commun.*, **59**, 377; 1974) is both transmissible to, and stable in, *P. aeruginosa*. The other point concerns the formation of the RP4-Nif plasmid. This was made in *E. coli* as the result of recombination between an F' Nif previously produced by Cannon, Dixon and Postgate (*J. gen. Microbiol.*, in the press) and the R factor RP4. In the present report Dixon, Cannon and Kondorosi suggest that this recombination occurred as a result of an interaction between identical translocating sequences carried on both plasmids. These sequences, corresponding to the carbenicillin resistance determinant on RP4 (TnA) and the same resistance determinant on the F', are capable of translocating on to a wide range of plasmids (Hedges and Jacob, *Molec. gen. Genet.*, **132**, 31; 1974). If the authors' suggestion that these translocating sequences can indeed facilitate recombination between plasmids that carry them, then they may well have demonstrated a general procedure for producing recombinant plasmids *in vivo*.

These studies would not have been possible without the availability of a group of R factors having the apparently unique property of being transferable to a very wide range of bacteria. The R factors, which all belong to the incompatibility group P (Datta *et al.*, *J. Bact.*, **108**, 1244; 1971), first appeared in the late 1960s in isolates of *Pseudomonas aeruginosa* found to be highly resistant to carbenicillin, a particularly efficient semi-synthetic penicillin for use in pseudomonas infections. Studies of these pseudomonads soon showed that in many of the isolates resistance to carbenicillin was plasmid borne (that is, R-factor mediated) and linked to determinants for resistance to tetracycline and kanamycin. Although at the time a number of plasmids were known in *Pseudomonas*, R factor medi-

Membrane nearest neighbours

from Howard Goldfine and Alan F. Horwitz

ALTHOUGH the series of twelve papers describing the total synthesis of the structural gene for a tRNA precursor in the February 10th issue of the *Journal of Biological Chemistry* is undoubtedly the more impressive achievement, two other papers from the laboratory of H. Gobind Khorana may be of even greater interest to those concerned with membrane structure and function. These papers describe the synthesis of various photosensitive fatty acids and phospholipids and provide evidence that these compounds can be incorporated into natural biological membranes. These new analogues should prove useful as membrane probes to study previously inaccessible interactions between lipid and protein in the membrane.

Chakrabarti and Khorana (*Biochemistry*, **14**, 5021-5033; 1975) describe the relatively easy four-step synthesis of azidostearic acids from commercially available hydroxystearate isomers. By standard synthetic methods they have put these and other photosensitive fatty acids on the middle carbon of the glycerophosphate backbones of phosphatidylcholine and phosphatidylethanolamine, both with palmitate esterified at the C-1 position. When the phospholipids were dispersed in an aqueous medium as sonicated vesicles and irradiated at 253 nm for 1 hour, extensive photolysis and cross linking occurred.

Greenberg, Chakrabarti and Khorana (*Proc. natn. Acad. Sci. U.S.A.*, **73**, 86-90; 1976) now report that an unsaturated fatty acid requiring mutant of *Escherichia coli* utilises the series of azidostearic acids with the azido group at various positions along the hydrocarbon chain. Most of these compounds were capable of supporting growth to the extent observed with the common unsaturated fatty acid, oleic acid. 12-Azido-oleic acid, a 16-azido *trans*-hexadecenoic acid, and two fatty acids with a bulkier 4-azido-2-nitrophenoxy group were also active, but the azidonitrophenoxy group on oleic acid did not permit growth at or above 25 °C. These studies show that the azido fatty acids are incorporated into cellular phos-

pholipids to the extent usually observed with *cis*-monounsaturated fatty acids. Furthermore, they were incorporated largely at the middle carbon of the glycerophosphate backbone as is usually the case with unsaturated fatty acids. Since the photosensitive fatty acids behave like unsaturated fatty acids, the MIT group looks forward to being able to study the interactions of lipids with functional membrane proteins.

When irradiated the azido groups form highly reactive intermediates, which attach covalently to nearest neighbours. This cross linking should provide information on the amino acid residues in close apposition to phospholipids, and since the photosensitive group can be placed near either the carboxyl or methyl terminus of the fatty acid chains, it should be possible to determine the depth of penetration of proteins into the bilayer and to map the exposed amino acid residues as well.

Reconstitution of membrane proteins with specific photosensitive phospholipids provides an alternative means of determining such spatial relationships. The polar head group of the lipids is another potential site for lipid-protein interaction, and two affinity labels have already been synthesised by Chakrabarti and Khorana, in which a polar photoactivable group replaces ethanolamine.

Extension of their work with an *E. coli* mutant to a similar mutant of a *Pseudomonas* species is already in progress, and they visualise future work with *Neurospora* and yeast mutants and even with mammalian cells grown in fatty acid deficient media. Although not specifically mentioned, questions of lipid-lipid "nearest neighbours" and membrane lipid asymmetry can also be approached with these compounds in either natural membranes or artificial systems. One caution, however, is noted. The azido group absorbs weakly in the ultraviolet and there is obvious overlap with nucleic acid and protein absorption. The use of radioactively tagged azido fatty acids may permit shorter irradiation times but other more photoreactive groups are being sought.

ated drug resistance in these exceptionally antibiotic-resistant bacteria had not been reported. The most unusual property of these R factors, however, was their ability to transfer from *P. aeruginosa* to *E. coli* (Sykes and Richmond,

Nature, **226**, 952; 1970). Although the publications that have followed this original observation suggest that all Gram-negative bacteria can act as recipients, as yet no report of transfer by conjugation or transformation to a

Gram-positive bacterium has appeared. Whether this represents a true difference in the ability of either group of bacteria to allow the replication and maintenance of plasmids from the other group, or simply that conjugation cannot occur and transformation has just not been reported, remains to be elucidated.

Future research using P-group plasmids carrying Nif or other genes looks promising, both for studies of gene function in different environments and for the genetic analysis of Gram-negative bacteria. However, problems of instability and the reduction in the host range of such plasmids, as reported by Dixon, Cannon and Kondorski, will need to be overcome. Also it has yet to be demonstrated that the type of recombination event involved in the formation of the RP4-Nif plasmid will provide a general method for making R' factors in the future. □

PETRA : come and help

from David J. Miller

PLANNING the future of European high-energy physics is not a very cheerful exercise just now, but there is one major ground for optimism, despite the budget squeezes which seem to be happening all round. The Germans have announced that they will go ahead rapidly with the construction of their electron-positron colliding-beam accelerator—PETRA. This machine, which should begin operations before its American equivalent, called PEP, is very similar to the EPIC project which Britain recently decided to abandon. It gives European physicists a chance to play a leading part in understanding the "new physics" which has emerged with the discovery of weak neutral currents and of the J/psi family of particles.

In deciding to build PETRA, Germany has shouldered a very heavy financial burden and it has been made clear that she cannot, by herself, afford to provide the large detection systems which such a machine will need. International collaborations are therefore being encouraged to bring equipment, expertise and manpower to join in the experimental programme. As a first houseparty to introduce possible collaborators to one another, the European Committee on Future Accelerators (ECFA) sponsored a meeting during the first week of March at the Frascati laboratory near Rome. It was attended by physicists from universities and laboratories throughout Europe, the United States and Japan. Theoretical

predictions were reviewed, confirming the enormous range of worthwhile physics available to the machine; experimental techniques were considered, and the basic parameters of PETRA were presented. The meeting became more political during the later part of the week when people put forward specific ideas for experimental equipment, and possible collaborators began discreetly to have lunch together. Professor Schopper, head of the laboratory where PETRA will be built, warned of the three essential steps in setting up a "marriage" between collaborating groups. First it is necessary to recognise the beauty of the prospective partner—how good are their ideas? This is usually clear on brief acquaintance, but the second question is more delicate—do they have money, or rather, do their parents (their governments) have money? Finally, one has to judge whether the collaborating groups are likely to be compatible throughout three or four years of hectic collaboration, with many crises to be survived.

A large proportion of the first sketches of apparatus for PETRA looked almost identical. The classic general purpose detector has a superconducting solenoid magnet with diameter and length between $1\frac{1}{2}$ and 6 metres. It is filled inside with drift chambers to reconstruct charged-particle tracks coming from e^+e^- collisions, and it is surrounded by electron, photon and muon detectors. Other schemes were also suggested which laid greater stress on detecting electrons and photons in particular, or on muons, or on recognising the exact identity of strongly interacting particles. The British contingent, representing half-a-dozen universities and the Science Research Council laboratories, presented a general purpose solenoid detector which had been costed more carefully than many of the other systems discussed. The estimated price is about £2 million, and it

is unlikely that all of this could be found from Britain alone since it would consume too large a fraction of the total national funds available for experiments at CERN and during the last years of NIMROD. In fact, none of the nations represented at the meeting seemed keen to pay for the whole of such a device by itself.

Courtships begun at Frascati must progress quickly. In order to have equipment ready for the first 15 GeV PETRA experiments in 1979 it will be necessary to start building the apparatus in the autumn of this year at the latest. The machine will eventually be able to accommodate six separate detectors, each enclosing a collision point of the two beams, but only two collision points are to be available at first so there will be strenuous competition to win one of these places. The British group, backed by the expertise of the Rutherford Laboratory in superconducting magnets, seemed to be among the more popular debutantes at Frascati. If the SRC is able to provide a respectable dowry, commensurate with expenditure on individual CERN experiments, then there is a good chance that a match can be made with one or more of the strong European groups, and that Britain will take part in e^+e^- physics in 1979 despite the loss of EPIC.

The strong, weak and electromagnetic interactions are so fundamental to all modern science and technology that we often behave as if we understood them, merely because we can use them. But major changes in our understanding are now taking place, and no-one doubts that results from both CERN and PETRA will be important in consolidating these ideas. European collaborations have played a major part in the first wave of the "new physics". Even in a time of economic troubles it would seem foolish for any government to allow its physicists to lose touch with this frontier of knowledge. □

New view of the cell cycle

from Robert Shields

THE period between successive cell mitoses is conventionally divided into four phases—G₁, the period between mitosis and the onset of DNA synthesis, S the period of DNA synthesis, G₂ the time between completion of DNA synthesis and the beginning of mitosis itself. Measurements of these phases show that whereas G₂, M and S are of relatively constant duration G₁

can attain widely different values depending on the conditions. Even within cell populations G₁ is usually highly variable in duration and the distribution of intermitotic times (times between successive mitoses) is invariably skewed towards longer time intervals. The conventional explanation for this skewness has been that it is some kind of reflection of hetero-

geneity in the cell population, but the reason why G_1 is much more variable than the rest of the cycle has never been adequately explained.

A new concept of the cell cycle has recently been proposed which not only predicts that cell cycle times must be heterogeneous but accounts quantitatively for their observed frequency distributions (Smith and Martin, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1263; 1973). The model divides the cell cycle into two parts. One termed the B phase is regarded as a deterministic and highly coordinated process; it corresponds to the conventional S, G_2 , M and part of G_1 phase. The other part (contained within G_1) is called the A state and is a sort of limbo in which the cell is not progressing towards mitosis; the cell may remain in this state for any length of time subject to the condition that under steady state conditions the probability per unit time (termed transition probability) of the cells leaving the A state and entering the B phase is a constant. In other words, cells leave the A state exponentially with respect to the time they have spent in that state in much the same way as a radioactive material will decay at a rate determined by its half life. In a population of cells the shortest intermitotic time is then given by the cell that leaves the A state immediately it enters, intermitotic times greater than this minimum should be exponentially distributed. The theory can account satisfactorily for the distribution of intermitotic times in a wide variety of cell types from bacteria to animal cells. What is more remarkable is that the theory predicts that the difference in intermitotic times of sibling cells should also be exponentially distributed (Minor and Smith, *Nature*, **248**, 241; 1974). This is observed in practice.

In principle the transition probability can take any value, high values would mean that most of the cells spend very short times in the A state, have fairly short homogeneous intermitotic times and so grow relatively rapidly. Low values would mean that the G_1 period would in the main be long or equivalent to so-called G_0 periods postulated for slowly or non-proliferating cells. Viewed in this way the problem of changing growth rate reduces to one of changing transition probability.

The paper by Brooks in this issue of *Nature* (page 248) examines the relevance of transition probability to mitogenic stimulation by serum of so-called G_0 cells. Since the characteristics of this type of stimulation are shared by systems as diverse as lectin-stimulated lymphocytes and liver regeneration after partial hepatectomy, the experiments may be relevant to other

systems as well. When serum is re-added to serum-starved fibroblasts there is a characteristic lag (which is independent of serum concentration) before any increase in DNA synthesis is seen. After this lag cells begin making DNA asynchronously. In a previous publication (*J. cell. Physiol.*, **86**, 369; 1975) Brooks showed that the apparent asynchrony in DNA synthesis was a result of the cells entering S phase with first order kinetics (that is exponentially), the actual rate constant depending on the concentration of serum used. Initiation of DNA synthesis can then be regarded as a random event occurring with a transition probability determined by the serum concentration. What serum does is to change abruptly the transition probability from the low value seen in quiescent fibroblasts to a higher value and so cells will leave the A state and enter the B phase (and hence DNA synthesis) at a faster rate.

The important aspect of these results is the observation that suboptimal doses of mitogen do not commit a subfraction of the population to enter DNA synthesis at a fixed time but cause the entire cell population to enter S phase with slower first order kinetics. The concept that a fraction of cells is committed by suboptimal doses of mitogen can now be seen as erroneous because it fails to take into account the kinetics of entry into S phase. Furthermore, the new value of transition probability produced by the serum is constant. This shows that mitogens such as serum do not trigger a sequence of events leading to DNA synthesis, for if they did, with increasing time after stimulation it would be more and more likely that the putative sequence was completed and hence the probability of entering DNA synthesis would increase with time. This is not the case.

In the present publication Brooks approaches the problem of the lag before any marked increase in DNA synthesis is seen. By following the kinetics of the entry into DNA synthesis after switching the serum concentrations from low to high values and *vice versa*, he comes to the conclusion that the lag after mitogenic stimulation represents the time required to effect any upward change in the transition probability regardless of its initial value and not the time taken to irreversibly commit all the cells to DNA synthesis.

It seems that the concept of there being a probabilistic transition within the cell cycle not only accounts for the heterogeneity of cell cycle times in a cell population, but positively predicts that such heterogeneity exists. Furthermore, the theory can account

satisfactorily for the kinetics of entry of cells into DNA synthesis after mitogenic stimulation. The biochemical basis for the probabilistic transition remains obscure, however, but it is only through an appreciation of the dynamics of the cell cycle that its nature will be elucidated. □

Pop goes the weasel

from a Correspondent

MOST mammalian carnivores tend to be fairly choosy about what they eat when food is plentiful but rather less so when it is not. An ability to cope with a wide range of food species has a high survival value for weasels (*Mustela nivalis*) which depend on characteristically fluctuating rodent populations. Brown and Lasiewski (*Ecology*, **53**, 939; 1972) have drawn attention to the difference in size between males and females and to the energetic problems their shape causes. The long, thin body is costly to keep warm and to compensate, weasels have a metabolic rate between two and three times higher than that of other mustelids (Iverson, *J. cell. comp. Physiol.*, **81**, 341; 1972). Not only are they territorial, thus reducing intra-specific competition for food, but they quickly reduce their reproductive rate should food become scarce (Erlinge, *Oikos*, **25**, 308; 1974) and, additionally, demonstrate a special kind of prey switching behaviour (Erlinge, *Oikos*, **26**, 378; 1975).

In spruce woodland in southern Sweden, weasels feed on whatever prey is available. A slight seasonal change in diet is observed with fewer young rabbits taken during the winter when they are less abundant. Other changes broadly reflect fluctuations in the rodent populations. Erlinge's data indicate that during spring and summer, when rabbits are relatively abundant and rodents relatively scarce, male weasels, which weigh almost twice as much as females, switch their attention to rabbits, leaving more of the rodents available to the smaller females. Rabbits constitute 40% of the males' diet in spring, with *Microtus agrestis* accounting for 35%; at the same time of year the latter species constitutes 86% of the diet of female weasels and rabbits just 6%. Similarly, males prey quite heavily on the large vole *Arvicola terrestris* and leave the bank vole *Clethrionomys glareolus* for the females. During autumn when rodents are abundant such segregation does not occur. Although Erlinge has shown that weasel populations are sometimes limited by food shortage, prey switch-

ing presumably allows the persistence of higher overall numbers.

British weasels are larger than their Swedish cousins but still show a marked difference between the sexes. Their catholic approach towards food has been demonstrated most recently by Moors who observed their feeding habits on two farmland study areas in north east Scotland (*J. Zool., Lond.*, **177**, 455; 1975), and found a similar situation to that in Sweden. *Microtus agrestis* and rabbits were particularly favoured items. But there was one marked difference between the studies which is as interesting as it is difficult to interpret. *Clethrionomys glareolus*, highly favoured by the Swedish female weasels was almost totally shunned by Scottish weasels of both sexes, yet on one of the two study areas bank voles were actually more abundant than *Microtus agrestis*. The little work done on the diet of British weasels does suggest that nowhere is *Clethrionomys* a food species of major importance, although it is very abundant (Day, *J. Zool.*, **155**, 485; 1968, Walker, *J. Zool.*, **166**, 474; 1972). Are British habitats in general and Scottish farmland in particular that much more favourable to weasels than southern Swedish spruce forests in producing sufficient numbers of the favoured and, perhaps, the less abundant rodent species? If they do, the size differential which has been shown to facilitate prey switching behaviour in Sweden is of lesser survival value to British popula-

tions. It may, however, enhance survival in years when all species of rodent are in decline. Further studies on weasel feeding ecology will be of the greatest interest to population ecologists and evolutionary strategists alike. □

Galactic gas dynamics

A meeting on Galactic Gas Dynamics was held by the Royal Astronomical Society in London on February 13, 1976.

from a Correspondent

THE principles of fluid dynamics can be applied with quite remarkable generality to problems in astrophysics. At the meeting astronomers learnt of the great versatility of gas dynamics in coping with the varied conditions in astrophysical plasmas. Within the basic theme of Galactic Gas Dynamics, we saw how ideas of fluid flow have been applied to plasmas with vastly different densities and scale sizes, from hot gas clouds occupying clusters of galaxies, to the problems of star formation.

Astrophysicists have long been intrigued by how such well-defined and apparently long lived spiral structures such as our own galaxy can exist without winding up in a disk of gas and stars that is rotating faster in its central regions than at the edge. The modern view is that these spiral patterns, or density waves, may persist as stars and gas move through them, and that a gravitational torque is developed, continually driving angular momentum outwards from the centre of the galaxy. According to V. Icke (Institute of Astronomy, Cambridge), gas has a particularly important role in the transport of this angular momentum. The more gas in a galaxy, the easier this transport becomes and a relatively open spiral results. He believes that this is the probable cause of the observed correlation between the proportion of gas in a galaxy and its classification in the famous Hubble Sequence of spiral galaxies.

T. Matsuda (University College, Cardiff) then spoke on the case of the peculiar 'barred' spirals, in which a rod-like structure or 'bar' is seen in the central regions. He considers that the complications introduced by this asymmetrical potential make the problem into a case for numerical techniques. The resulting computed flow patterns show that a suitably shaped bar can indeed set up a two-armed spiral flow, with shock fronts that agree well in position with regions of star formation

seen in actual galaxies. What did not emerge from the calculations, however, was why such a bar should have formed in the first place.

The afternoon was devoted to smaller scale galactic phenomena, the formation of stars and HII regions and the interaction of supernovae with the interstellar medium. With the recent widespread use of numerical techniques, F. Goldsworthy (University of Leeds) sounded a cautionary note with regard to the treatment of ionisation fronts, the sharp boundaries between neutral and ionised hydrogen surrounding young, hot stars. Unlike the relatively simple shock fronts that occur in ordinary gas dynamics, an ionisation front can be of two species, each with two strengths, and one of these, the strong 'deflagration' front, is characterised by an inherent indeterminacy difficult to overcome in computer solutions. Goldsworthy reported on a noble attempt to exorcise this particular devil by means of a scheme that at least manages to avoid its invocation.

S. Falle (University of Manchester) made a similar point about the usual numerical method of treating shocks by including an artificial viscosity term. He showed that this method can be greatly in error if used to estimate the luminosity of the radiating shocks that occur in the late stages of supernovae. By using more accurate shock-fitting techniques, Falle has so far managed to distinguish five separate types of old supernova remnant. On the other hand, F. Kahn (University of Manchester) was prepared to use an analytical approximation in his treatment of this subject, and reported that very substantial amounts of hot gas must exist in a supernova remnant, even a million years after the supernova explosion. At this late stage remnants are likely to bump into, and coalesce with, their nearest neighbours in the Galactic plane. He calculates that about 30% of the Galactic gas is kept hot in this way.

As the meeting progressed it became clear that an impressive array of numerical techniques is now available to tackle these gas dynamical problems. During the day the results were shown from two massive computer codes using two spatial dimensions and no less than four one-dimensional programs appeared. But the talk by A. Nelson (University College, Cardiff), who was concerned with the small scale warping or corrugation of gas in the Galactic plane, served as an illustration of one of the cardinal rules of gas dynamics. Fluid flows, no matter how symmetrical they might appear, are inevitably three-dimensional, and it will still be some time before numerical methods can catch up in this tricky subject. □



A hundred years ago

THE general staff of the German empire has published a report of experiments made in Germany on ballooning at the expense of the Imperial Government. The conclusions throw no new light on the subject, but the German officers believe that the mechanical direction of balloons is by no means an impossibility. They even suppose that the problem of ascending or descending without using ballast or the valve, is very likely to receive a speedy solution. They propose to the Government to determine by means of experiments what is the best diameter for the helix when it is applied to a balloon of a certain capacity. They propose also to try the efficacy of wings for propelling balloons. They are not of the common opinion that the diameter of balloons can be indefinitely enlarged.

from *Nature*, 13, March 16, 397; 1876

Book review supplement

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Male Kittiwakes contesting a cliff nest site

Photo: Niko Tinbergen

IN his introduction to a volume of Tinbergen's collected works published a few years ago, Sir Peter Medawar recounted the story of an eminent neurophysiologist who said of ethology "Why, that's just bird-watching, isn't it?". The point of the story is, of course, that Niko Tinbergen has done more than any other single person to transform the biological study of behaviour from descriptive natural history into a hard experimental science. After emphasising description as a starting point, Tinbergen and his associates have succeeded in carrying out simple, but rigorous and incisive, experiments in the laboratory and field to elucidate problems ranging from why seagulls are white, to how digger wasps find their nests.

Five years ago, I think that most ethologists would have agreed that the most exciting developments in what might broadly count as ethology involved the meeting point of ethological and neurophysiological research—for example, in the mapping of neural pathways underlying simple behaviour patterns in molluscs. In the last year or two, however, things have changed, and there has been a dramatic upsurge of interest in the area in which Tinbergen made some of his most important contributions. We used to call it behavioural ecology, but now it is known as sociobiology. Sociobiology (as defined by the contents of E. O. Wilson's book) involves much more than the work of Tinbergen and his students, but the studies carried out by his group are without doubt among the best in elucidating how natural selection acts to mould the social organisation of a species. Work on the adaptive significance of colonial nesting in gulls, and on the social organisation of carnivores are examples which spring to

mind as typifying the blend of ecology, behaviour and evolution which characterises much of the work of the Tinbergen group.

In the past, ethologists as a whole can be faulted for having been somewhat careless in thinking about how natural selection influences behaviour, and it is a pity to see the confusion continue in this volume dedicated to one who thinks so clearly about the problem. There seem to have been

Analysis of function

John R. Krebs

Function and Evolution in Behaviour. (Essays in Honour of Professor Niko Tinbergen, FRS.) Edited by Gerard Baerends, Colin Beer and Aubrey Manning. Pp. xxxi+393. (Clarendon: Oxford, Oxford University: London, February 1976.) £16.50 net.

three types of difficulty in the literature dealing with the function or survival value of behaviour, all of which occur in this volume of essays. The first, and most widely appreciated, is the old Panglossian fallacy that natural selection favours adaptations that are good for the species as a whole, rather than acting at the level of the individual. This type of argument crops up in the article by Immelmann on early learning—he suggests that imprinting is advantageous because it enables a species to evolve rapidly—as well as in the article by Baerends in which he makes a similar case for the advantage of flexible motivational control displays. The second 'function' fallacy is discussed at length in articles

by Hinde and Beer. It is partly a semantic issue arising from the oft-posed question "What is the function of such-and-such behaviour?", which is of course shorthand for asking why natural selection, the differential survival of genotypes, favours that particular behaviour. The problem with this question is, as Beer and Hinde point out, that most behaviour patterns (or structures) are likely to have several functions. Territorial behaviour may increase fitness because it preserves a food supply near the nest, it reduces the chance of predation and so on. The confusion arises when one tries to ask which function came first, an historical question which cannot be answered by 'survival value' experiments. Sometimes it is possible to deduce which function came first on logical grounds or on features of design. Thus one can conclude that any population-limiting effect of territorial behaviour is likely to be secondary; and the design of a reddish egret's (*Dichromanassa rufescens*) wing suggests it is primarily a locomotor organ, although it may be held outstretched in feeding, to cut down reflection from the water surface. The use of its wing in feeding, however, increases a reddish egret's chance of survival, so this has to be counted as one of the functions of wings. It is difficult to see how one can distinguish, as Hinde claims in his essay, between 'functions' and 'biologically advantageous consequences which change reproductive success'. It is clear that function is, to quote G. C. Williams, an "onerous concept", so perhaps the best solution would be to rephrase the question mentioned above as "How does such-and-such behaviour influence fitness?"

The third difficulty in the analysis of function is that ethologists often make

the implicit assumption that behaviour is more responsive than are other features of an animal to the action of natural selection. To give an example, it is a widely accepted ethological fact that many courtship and threat displays in which the participants suddenly face away from each other, serve the function of cutting off stimulating visual input and so allow the animals to control their motivational state. Another familiar example is the 'Fraser-Darling Effect': the idea that colonial nesting and communal displays function to enhance gonad growth through mutual stimulation. Both of these arguments imply that natural selection is not very good at designing animals with efficient physiological systems, so that it has to call on behavioural adaptations to sort things out. This is not to say that the cut-off hypothesis is wrong, but without careful thought it is all too easy for the ethologist to dream up glib functional explanations for behaviour by assuming that other features of the animal are not subject to natural selection as well.

In addition to the two general discussions of function, to which I have already referred, the book contains several more specific studies of the effect of natural selection on behaviour. Of special note are the papers by Liley and Seghers who show how predation pressure influences morphology and behaviour in guppies, Kruuk on the relationship between social organisation and feeding habits in carnivores, and Patterson's attempt to disentangle the factors influencing fighting in rook flocks. Baerends reviews the literature on the motivational and evolutionary origin of displays, and the overall impression is that little progress has been made since Tinbergen wrote his major paper on this subject in 1959. Although it does not seem to have filtered through to the ethological community, I think it likely that the recent game theory analysis of ritualised conflicts, by Maynard Smith and Parker, will provide a starting point for totally new insights into the nature of courtship and threat displays.

Without listing the contents in detail, I think it is fair to say that, although there are good discussions of various areas of important progress in ethology as a whole, the book does not reflect the exciting new developments—for example, in the quantitative analysis of function—which have arisen specifically out of the lines of research initiated by Tinbergen himself. Perhaps by including contributions from some of Tinbergen's recent students and collaborators, the volume could have given a better impression how his particular inspiration is as much as ever at the very forefront of ethological research.

Description before analysis

Roger French

The Great Instauration: Science, Medicine and Reform, 1626–1660. By Charles Webster. Pp. xvi+630. (Duckworth: London, November 1975.) £13.50.

"THE history of science has predominantly assumed the character of an index of progress towards our present intellectual position". With these opening words, the author proceeds to justify an examination of science in seventeenth century England, and to criticise the growth of a rather whiggish internalism in historical accounts of the sciences. Now, one of the reasons for regarding history of science, and in particular, history of medicine, as an independent discipline, is because the evidence to which the historian first turns his attention is of the same nature as that presented to the figures he is studying—that is, textual evidence—whereas the historian-at-large is faced rather more often with written evidence of something that is not documentary, like a political action. The directness, continuity and the subject matter itself of the former lead naturally to a history-of-ideas approach, whereas the inferential nature

of the latter directs the attention to the historical circumstances of the object of study. Those with an interest in the history of science know that the historian of ideas can sometimes neglect every other historical factor, and that on the other hand a circumstantial history can degenerate into the merest sociology. By and large, history of medicine at least is underworked. We should resist the temptation to frame hypotheses and find causes in history before we are done with the texts that furnish us with the historical evidence. Description should precede analysis.

The study of seventeenth century English science has many pitfalls of this kind. Although his book is 'history-at-large', Webster falls into none of them. He makes us aware that historical circumstances in the sense used above can be intellectual, and the plan of his book is to reveal the progress of the *idea* that man can restore himself to the state of knowledge of, and command over, nature that he possessed before the Fall. In working out this idea, attention was given to education, to agriculture, to public health, and above all to Reform. Webster examines in considerable detail the social, political and religious groupings of the exponents of these ideas in a way that adds another dimension to the more usual and more technical accounts of the Baconians and Harveians of the English seventeenth century. So consistently is this approach followed that it disarms the critic who might expect a technical exposition of Harvey's work in a book with this title. Webster also reminds us that description should precede analysis in not attempting too great a correlation between the results of the internal and external approaches. It would be nice to know why Culpeper felt that his reforming zeal could best be served by translating the traditional, rather scholastic and largely discredited *Ars Medica* of Galen. Indeed, Culpeper's whole notion of medicine was Galenic, as was that of the College of Physicians whose monopoly he attacked. It would also be nice to know more precisely the relationships between the professional institutions of the physicians on one hand and on the other the internal changes in Galenism that led to its retention as a doctrine, or to its collapse. Webster's exploration of the intellectual and social context of the 'most intrinsically interesting' scientific developments, the examination of 'the worldview of a particular society' is pursued without the distortion caused by the spectacles of modern science, but with a wealth of detail that will make the book invaluable to historians of all shades and the natural authority for those working in the period. □

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Riding roughshod over cancer research

John Cairns

The Biology of Cancer: A New Approach. By P. R. J. Burch. Pp. 452. (MTP Lancaster, January 1976.) £11.50, \$23.23.

PROFESSOR BURCH has recently acquired a certain fame thanks to his much publicised assertion that cigarette smoking is not a cause of lung cancer. This is just one consequence of his theory that most diseases are largely determined by random events within us that owe nothing to external agents in our environment. He has now written a detailed account (one might call it a polemical tract) describing how he came to his present, unorthodox position.

The argument runs as follows: Over most of our lifespan the incidence of the common cancers rises roughly as the fifth power of age. This is usually explained by saying that cancer is the end result of several independent steps (mutations) whose frequency among the cells at risk accumulates linearly with time. On further scrutiny it turns out, however, that many other diseases—ranging from arteriosclerosis and osteoarthritis to certain forms of psychosis—show a similar relationship to age. As these diseases are clearly multifocal in origin, they can hardly be ascribed to a particular set of rare mutations having occurred in one of the cells at risk in artery, joint, brain, and so on. If we want to persist with multistep models for disease, we should therefore try to come up with some formulation that allows a set of mutations in one cell to be the cause of unifocal or multifocal changes among other cells. So let us postulate that the main tissues of the body are divided up into a large number of subsets containing a few hundred cells whose growth is controlled by a central system of cells divided into a similar number of subsets, and let us postulate that most diseases are due to defects in the central controlling cells; unifocal diseases like cancer are due to mutations that fall within a single subset of controlling cells; multifocal diseases like arteriosclerosis are due to mutations in the primordial stem cells which are the parents for many subsets of controlling cells. Burch goes on to suggest that the central controlling cells are lymphocytes, that the dialogue between central and peripheral systems



Professor Burch, Photo: Leeds University

is conducted by means of pairs of specifically interacting molecules, and that these molecules are coded for by genes which contain palindromic base sequences and therefore are apt to be read backwards as well as forwards.

The theory owes an acknowledged debt to such notions as the control of growth by 'chaperones', the recognition of self and not-self, the creation of 'forbidden clones' and the control of cancer by immune surveillance—ideas championed by many people, Burnet in particular, at one time or another. As formulated by Burch the theory provides a plethora of independent variables—the mutation rates for the particular genes involved, the number of genes that must be altered to inactivate an appropriate central controlling cell, the number of such cells that have to be affected, the proportion of people that are genetically susceptible, and so on. Because some of the functions that can be generated with the model do not increase steadily with time but go through a maximum, the theory can be made to take in even such things as the age distribution of a childhood disease like measles (for me, the clinching *reductio ad absurdum*). Further, as Burch modestly points out, "so far as I am aware, our theory is the only one that offers a general solution to the problem of the anatomical specificity of the lesions of natural disease".

Now, all this curve fitting would be just innocent fun, albeit of a rather old-fashioned kind, were it not that somehow the idea of chance events scattered randomly among central controlling elements has been taken to mean that the events cannot be due to external agents. I must say I do not understand the logic here; after all, one of the first collections of biological data shown to follow the Poisson distribution was the number of men killed by horses each year in the Prussian army, and there the external agent was plain for all to see, on the hoof

as it were. Anyway, that is what Burch believes and so he sets out to show that the incidence of the common cancers is not perceptibly influenced by environment and therefore that nothing can be done to prevent cancer, or most other diseases if it comes to that. (I might sympathise with this attitude if he were an old scientist subconsciously wishing to make the virtues and rewards of science decline to zero coincidentally with his retirement, but Burch is hale and hearty as far as I know and has no reason to go round preaching a kind of molecular nihilism).

Obviously the theory rides roughshod over most forms of cancer research. For example, the connection between external agent and susceptible cell is too direct to be denied for the various experimental cancers in animals and for most industrial cancers, and so Burch is forced to say that these are exceptional and have no bearing on the common 'natural' cancers. As for the copious epidemiological evidence that the incidence of the common cancers changes with alterations in habits and environment, he argues for a combination of illusion and conspiracy—for example, Japanese migrants to the US show altered cancer rates because they have a genetic constitution that drives them to migrate to a country where coincidentally the population suffers the same spectrum of cancers as they themselves; similarly, the apparent steady decline in stomach cancer and rise in lung cancer over the past 50 years are artefacts due to changes in diagnostic fashions.

Now, I believe that all this is what might euphemistically be called wrong. Indeed, I think that even Burch will come round to that opinion. Were the biology of cancer a normal branch of science, his unorthodox beliefs might be stimulating and would add a certain piquancy to the lives of cancer research workers, like the pleasure one gets on meeting someone who really believes the earth is flat. Unfortunately the biology of cancer is not that kind of subject. This book will undoubtedly persuade many people to continue smoking who might otherwise have stopped, and may confuse those who legislate on the search for and control of carcinogens in our environment. Some of these people will, I am sure, have been persuaded not by actually reading the book and being swayed by the inexorable logic of his argument, but simply by the thought that when a professor writes a fat, obscurely mathematical tome on any subject whatsoever, there must be something in what he is saying. And that is why I wish this book had not been written. □

Genial corrective

A. R. Ubbelohde

The Royal Society and Its Dining Clubs. By T. E. Allibone. Pp. xv+457. (Pergamon: Oxford and New York, 1976.) £10.

EFFECTIVE pursuit of natural science demands much concentration. Dr Johnson once compared a course of chemistry in this respect with a course in walking the tight-rope, as a cure for Boswell's melancholy. Clearly, a Dining Club provides genial correctives for any excessive abstraction scientists may have to practice. This adds a distinctive relish to the long history of 'clubbable' Fellows of the Royal Society dining together. Dr Allibone's narrative is the present successor to earlier chronicles of the Royal Society's Dining Clubs. Two of these histories, by Sir Archibald Geikie and Dr Bonney only stretch down to 1901, so that Dr Allibone has had to grapple with some most memorable events in science as well as in world affairs. From his 'history' it is entertaining and fascinating to mark the uneven penetration of such events into the after-dinner discussions of the Club.

At the present time, the club numbers rather less than 100 members. Only a fraction come to dine on any one evening. It is also the custom for individual members of the Club to bring distinguished visitors, often from overseas, to a dinner. Quite often the President of the Royal Society is in the chair. Under the Chairman's guidance the talk ranges informally, over narrow specialist interests as well as widely in relation to the world at large. Week by week and year by year the Senior Treasurer for that year keeps records of those present, their dinner menu, and the heads of discussion, for storage in the club archives, which are kept private until the next history is due to be written.

Much praise is due to Dr Allibone for the way in which he has tackled this tremendous task. He makes it clear how formal continuity of the present Club can be traced back to 1743; but the Club can claim plausible links with earlier diners up to 1645. Samuel Pepys records his evident satisfaction with the fare as well as the discourse in the seventeenth century. Now, the club fare has become decidedly 'plainer' even if our thinking is 'higher'. It may be some mitigation too of any euphoria at present meetings of the Royal Society that dinners of 'The Club' now follow these, instead

of preceding them as in Pepys' time.

With over 200 years of continuous archives to deal with, the present history has perforce concentrated much information in the form of nourishing pemmican, or sometimes by presenting well articulated skeletons from amongst the scientific worthies of the past. The long list of famous names cited makes the book valuable as well as fascinating. Different readers will probably let their imagination seize on different personalities to clothe, and Dr Allibone is to be congratulated that the choice provided is large. Thus it is striking to find that Henry Cavendish, who is often pictured as a millionaire scientific misanthrope, was one of the most assiduous diners. Clearly, a 'clubbable' man of his day. The colourful personality of Sir Joseph Banks receives rather lurid light from the record of his having purchased a silver bowl holding a gallon of punch and also of his having led a breakaway dining club into schism. When later he was elected President of the Royal Society, however, the schism was gradually healed. Then, too, there is the rather sad little note that Lavoisier in 1786 established a monthly dining club of scientists, the 'Philomatic Society', almost under the shadow of the guillotine.

This does raise the question (with one eye on Dr Johnson and perhaps one on Mr Pickwick) whether elite dining clubs (however chosen) are truly congenial only to the British temperament. One may hope that modern conditions of living will never extirpate such an amiable weakness or custom. So far as the Royal Society dining club is concerned, Dr Allibone's history will certainly help to perpetuate its remarkable longevity. □

Authentication in art

Harold Barker

Authenticity in Art: The Scientific Detection of Forgery. By Stuart J. Fleming. Pp. 164. (Institute of Physics: London and Bristol; Crane, Russak: New York, 1975.) £6.50.

THE majority of published books on authentication in the art world are concerned either with stylistic criteria or are purely anecdotal in character and do little to illustrate the significant contributions made by scientific methods of examination in this field, particularly during the past few decades. The author of this book, himself a scientist who has made notable contributions to the development of thermoluminescence dating which has revolutionised the authentication of

pottery, has set out to redress the balance and describe how scientific methods are applied to the detection of forgeries.

The book is divided into four main sections, the first of which provides a general introduction to the subject in which the author discusses the limitations of stylistic judgements; presents some early examples of the detection of forgeries; discusses different types of fake, the forger's technical approach and the errors and stylistic anachronisms which he is likely to perpetrate, and the role of surface examination by microscopy and ultra-violet fluorescence in uncovering forgeries. The other three sections are concerned with paintings, ceramics and metals, respectively. There is also an appendix devoted to X-ray fluorescence of Chinese blue-and-white porcelain, radio-carbon analysis, and lead isotope analysis of ancient objects.

These sections are concerned much more specifically with scientific techniques of examination covering many case histories and ending with fairly extensive bibliographies which deal adequately with case histories but could perhaps have benefited by the inclusion of at least a few more references to the fundamentals of the scientific techniques used for the benefit of readers who may wish to widen their background knowledge. For example it is rather surprising to find no reference to Zimmerman's development of the fine grain technique of thermoluminescence dating in the section on ceramics nor any reference to a basic metallurgical text in the section on metals. Also, in this age of high specialisation, the expert, when writing outside his own field, is not immune from mistakes and misconceptions, sometimes of an elementary nature. Thus in the section on metals, one discovers a statement (p125) which assumes that the production of tin bronze is associated with a significantly lower temperature technology than that of copper alone.

It is also inevitable that any publication concerning the authentication of valuable objects must be somewhat circumspect in its treatment of the material in order to avoid possible legal complications. Thus in its reliance on published examples in which the results of scientific examination are unequivocal, the book tends to give the impression that scientific techniques are rather more certain in the answers that they can provide than is actually the case. In general, however, and in spite of these limitations, the book is an excellent introduction to the scientific detection of forgeries and a good source of references to the literature of authentication and associated scientific studies. □

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Source of constructive thinking

Marcel Florkin

The Evolution of the Bioenergetic Processes. By E. Broda. Pp. vii+211. (Pergamon: Oxford and New York, 1975.) £8.30; \$20.

In this much needed synthesis about the sources of free energy and their evolution in organisms, Professor Broda has collected and integrated a vast body of knowledge. He begins with a detailed exposition of current views on the origin of 'eobionts' and considers as most ancient those bacteria which are able to make ATP (the universal source of free energy in metabolism) anaerobically in a solution of organic compounds without direct participation of light. Such 'fermenters' (found among *Clostridia* and among methane formers, both gram positive) could only depend on the chemical energy of solutes. As the biomass increased, critical conditions arose—the production of compounds with high energy content by radiations, and so on, falling behind the rate of consumption of redox compounds useful in metabolism. At this point additional sources of free energy were acquired by photosynthetic bacteria through emergence of photosynthetic phosphorylation: in light, useful carbon compounds were regenerated from waste, and life could persist on the basis of antagonistic dark and light reactions.

Photo-organotrophs such as purple non-sulphur bacteria use as electron donors in the light, in anaerobic conditions, those organic electron donors which were used by fermenters in the dark. In addition to ATP, the expansion of life required, for the assimilation of CO₂, an external supply of electrons. Photolithotrophs such as *Thiorhodaceae* or purple sulphur bacteria and *Chlorobacteriaceae* or green sulphur bacteria) learned, through photochemical promotion, to use inorganic reductants as electron donors.

Photolithotrophs are removed from fermenters by one more step than photo-organotrophs. As photolithotrophic bacteria never produce oxygen (they do not extract electrons from water) the atmosphere remained anoxygenic, but sulphate (a mild oxidant) became plentiful in the bio-

sphere. Oxygen increased in the atmosphere after the emergence of the ability of green plants (phytotrophs) to photolyse water and use it as reductant. Oxygen is toxic for anaerobes and it must be considered that a progressive oxygen tolerance was acquired by aerobic organisms who developed enzymes (oxygenases) through which oxygen was first exploited in biosynthesis. The author concludes that all respiring bacteria (relying on electron chains) have developed from photosynthetic bacteria and that the electron chains of respiratory phosphorylation were derived from the electron chains of photosynthetic phosphorylation.

It should be noted that non-photosynthetic lithotrophs (chemoautotrophs) as well as respiratory organotrophs, rely on oxidation in air to obtain ATP, resulting, in both, from respiratory phosphorylation, with the distinction that in the case of non-photosynthetic lithotrophs, the reductants are inorganic. The author also discusses the evolutionary positions of the variants of respiration (anaerobic and aerobic) in prokaryotes. He suggests that sulphate respirers (using sulphate instead of oxygen as the terminal electron acceptor in the electron flow mechanism characterising 'respirers') arose from some purple non-sulphur bacteria.

Bacteria and blue-green algae are prokaryotes; all other algae, higher plants, fungi, *Protozoa*, *Metazoa* are eukaryotes. Eukaryotes possess specific organelles (mitochondria, chloroplasts) housing the enzymatic machinery or respiration or photosynthesis. All eukaryotes are aerobic and are derived from eukaryotic protists (*Metazoa*, or higher animals, derived from *Protozoa*; *Metaphyta*, or higher plants, derived from green algae).

Professor Broda discusses the evolution of intracellular organelles in eukaryotes and the theories considering mitochondria and chloroplasts and endosymbiotes.

The book is completed by a discussion of the aspects of differentiation of tissues in the frame of oxygen need, that is, the further line of bioenergetic evolution among animals and plants. The author also considers the incidence of paleontological and geological evidence on the path of bioenergetic evolution deduced from the properties of extant prokaryotes and eukaryotes, and in the last chapter, retraces the history of atmospheric oxygen.

All students of evolution must be grateful to Professor Broda for providing such a lucid and documented discussion of this important topic. His book fills a gap in the literature on evolution and will certainly remain for a long time a source not only of bibliographic information, but also of constructive thinking. □

Photoreceptors

Aubrey Knowles

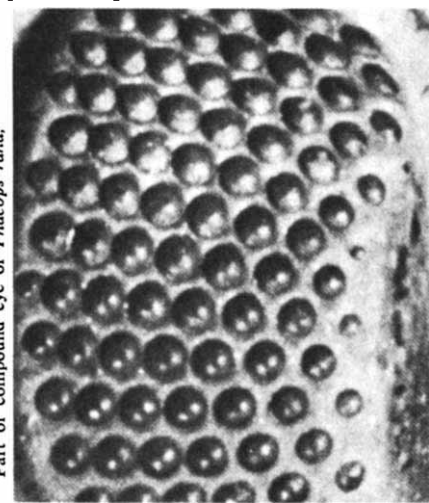
Photoprocesses, Photoreceptors and Evolution. By Jerome J. Wolken. (Academic: New York and London, November 1975.) \$19.50; £10.15.

I WAS sadly disappointed that the promises of the title of this book, and its preface, were not fulfilled. The rapid progress of research into photosynthesis, vision and other fields of photobiology would make an overview of the relationship of the sun to life processes most timely. Unfortunately, Dr Wolken's approach is seriously biased by his own interest in invertebrate photoreceptors, resulting in a book that is badly out of balance—for example, the vision chapters occupy 110 pages, photosynthesis is confined to 27, and evolution, although appearing in several section headings, is barely mentioned in the text.

At best, the book presents a survey of visual photoreceptor structures in vertebrates and invertebrates, but most of the information, including the illustrations, appeared in Dr Wolken's earlier monographs. Beyond this, the text is a mixture of wide-eyed statements about the wonders of nature, and a mass of unrelated and often misleading facts, making a book that is not only difficult to read but that has to be read with the greatest wariness. In his preface, Dr Wolken says that the book is not intended for experts, but I find it hard to recommend it to anyone without a fair knowledge of both photobiology and evolution: there is much that is irrelevant, out-of-date, and—more serious—much that is omitted; for example, a Jablonski energy level diagram (carelessly copied from another author) is included without any explanation; a page is devoted to the geological time scale, but this is not mentioned again; 10 pages are given to a short account of bioluminescence that seems to have no place in the text, for in the author's own words, "The relationship of bioluminescence to photoreception . . . in evolutionary development is far from understood"; and so on.

The book is nicely produced, but I am curious about the two-inch wide margins; are these intended to raise the book into the 'coffee table' category, or are they for the reader's own additions? I had hoped to learn more about the bearing of light on evolution, but beyond token references to Darwin, Mendel and Karl Marx, the only photobiological system discussed in evolutionary terms is vision. Even in this restricted field, the fascinating

story of the development of photoreceptors is barely mentioned, and we are given little more than a repetition of George Wald's 20-year-old theories on the evolution of visual pigments. There is no mention of the work of Bridges, Munz, McFarland and others over the past 10 years even though this has provided some hard facts about the relationship of visual pigments to environmental factors. In view of this poor treatment of evolution, and the sketchy information on primary photoprocesses, I suggest that the intending reader should ignore the first and third words in the title, and regard the book simply as a rather limited survey of photoreceptors. □



George Duncan

Photoreceptor Optics. Edited by A. W. Snyder and R. Menzel. Pp.x+523. (Springer: Berlin and New York, 1975.) DM 97; \$41.80.

THIS book comprises a collection of papers that were presented at a colloquium in October 1974. Any text that is published within nine months of the meeting that conceived it is indeed remarkable and *Photoreceptor Optics* is doubly laudable because its chapters are so cross-referenced and deftly edited that we are made to feel that the authors were interested and indeed stimulated by the papers of their coparticipants.

The text is concerned with "how the optical properties of photoreceptors—their arrangement, orientation, shape, size, refractive index and membrane properties—influence their absorption of light and establish many of their specialised functions", and the initial section gives the most comprehensive treatment available of the role of wave guide optics in photoreceptor processes. The excellent theoretical chapter by A. W. Snyder is essential reading for all who debate the reasons for the intrinsic directionality of photoreceptors, or the im-

portance of optical coupling between receptors.

After the mathematical rigour of the initial section, the second and third sections on photoreceptor membranes provides light relief, especially when introduced by a contribution by P. A. Liebman that manages to be both nutritious and easily digested. He provides a good review of the classical problem of determining the contents of photoreceptor cells by optical means, but he also gives us a glimpse of his very interesting present work on the axial diffusion of photopigment in cones. His findings go a long way to explain the difference in the distribution pattern of radiotracers in vertebrate rods and cones found by R. W. Young. He also focuses our attention on the small and hitherto unnoticed axial gradient of birefringence in rods, and on the basis of the observation, he spins a marvellous (and convincing) tale of the ageing of lipids in the rod-disc membrane.

Although the importance of pigment granule migration is acknowledged throughout this book, there is only one short paper on the possible photo-mechanical mechanisms at play, and that, by W. H. Miller, simply amplifies an earlier work by Miller and Cawthon. They suggest that microtubules mediate the movement of pigment granules in *Limulus* and they base their argument on the fact that the application of colchicine causes the same centripetal movement of shielding pigment as light does. It would be interesting in this respect for someone to repeat the experiments of W. A. Hagins who reported in a brief communication ten years ago that there was in fact no pigment migration when the illuminated photoreceptor (in this case cephalopod) was bathed with sodium-free medium.

The great complexity of the arthropod photoreceptor system is highlighted by G. A. Horridge in the concluding section. From an analysis of intracellular microelectrode data, he found that there were two discrete sizes of quantum bumps in cells 1–6 of the fly's ommatidium. The miniature potentials elicited by 333-nm light are in fact larger than those produced by 500-nm light incident on the same cell, and so it seems that there are probably two transduction mechanisms within the same cell. It also seems likely that one pigment lies above the other and therefore orthodromic illumination and antidromic illumination may be differentially absorbed.

Although this text is within reach of the corporate rather than individual pocket, the conscientious researcher in the field of photoreceptor optics should ensure that he has ready access to a copy. □

Classic work on astronomy

G. J. Whitrow

A History of Ancient Mathematical Astronomy. Part 1: Pp. xxi+1-555. Part 2: Pp. 556-1,058. Part 3: Pp. 1,059-1,456. (Studies in the History of Mathematics and Physical Sciences, Volume 1.) By O. Neugebauer. (Springer: Berlin and New York, 1975.) DM 290; \$118.90.

PROFESSOR Otto Neugebauer, of Brown University, presents us with a magnificent work that will surely become a major classic in the historiography of ancient astronomy, a three-volume treatise devoted almost entirely to the mathematical astronomy of the Babylonians and of the Greeks.

He begins by solemnly warning his readers that "Many things are omitted here. The reader who wants to read about Archimedes taking a bath or about the silver nose of Tycho Brahe can find innumerable books which dwell on these important, biographical matters . . .". Instead, Neugebauer deals exclusively with "numerical, geometrical and graphical methods devised to control the mechanism of the planetary system". He omits any discussion of the theory of instruments (and also of Chinese and Mayan astronomy, neither of which had the slightest influence on the work of those about whom he writes).

For about 1,500 years (from the second century AD to the seventeenth century) mathematical astronomy in Europe and Islam was dominated by Ptolemy's *Almagest*. Book I of Neugebauer's *History* is therefore devoted to that tremendously influential work and its direct predecessors. He begins with two chapters on the plane and spherical trigonometry known to Ptolemy (about 150 AD), chords being used instead of halfchords, or sines (a later invention of the Hindus), and absence of knowledge of the fundamental formulae for right spherical triangles being compensated for by ingenious use of an important theorem attributable to Menelaus (98 AD). There follow three chapters on spherical astronomy before we plunge into Ptolemy's lunar theory (pp53-144) and his planetary theory (pp145-261). The remaining 80 or so pages of Book I are devoted to Apollonius and Hipparchus. The most important question of attribution confirmed by Neugebauer's authoritative analysis, although originally attributable to H. Vogt (*Astronomische*

Nachrichten, 1925), is that the still widely held view that Ptolemy's star catalogue was largely based on Hipparchus's, a hypothesis accepted by the influential early nineteenth century historian of astronomy Delambre (because of his "preference for any theory which would detract from Ptolemy's merits"), cannot be sustained. This conclusion has been accepted by K. Lundmark, Owen Gingerich, and others.

Neugebauer is convinced that Hipparchus used arithmetical as well as geometrical methods. Just as the latter were the peculiar contribution of the Greeks, so the former, it now seems, (due to the researches this century by Kugler, Neugebauer, Van der Waerden, Aaboe and others) owed much to the Babylonians. Consequently, we can view Greek astronomy in what Neugebauer calls "an entirely new historical perspective", for we now realise that Babylonian arithmetical techniques greatly influenced both ancient Greek and also medieval astronomy. Book II (pp347-555) of Neugebauer's treatise is therefore devoted to Babylonian astronomy. A significant difference between the records of Babylonian and Greek astronomy available to us is that the former are authentic contemporary documents, dating from the last three centuries BC, even if some are copies of earlier tablets. Nevertheless, we have no information about the development of the completed system of Babylonian astronomy, unlike Greek astronomy. "In short", comments Neugebauer, "we probably know as much and as little about Babylonian astronomy as a Greek astronomer of the Hellenistic age knew". It is a mathematical astronomy that "operates without any model of a spherical universe, without circular

motions and all the other concepts which seemed 'a priori necessary' for the investigation of celestial phenomena". It is entirely arithmetical.

Book III, only 12 pages long, is on Egyptian astronomy, the only important contribution of the Egyptians being the 'Egyptian year' of a fixed length of 365 days. It was the 'backbone' for the computation of astronomical tables down to the Renaissance.

Book IV (pp571-776) is concerned with pre-Ptolemaic Greek astronomy. I cannot resist quoting a short passage from page 572: "I see no need", Neugebauer writes, "for considering Greek philosophy as an early stage in the development of science . . . One need only read the gibberish of Proclus' introduction to his huge commentary on Book I of Euclid's *Elements* to get a vivid picture of what would have become of science in the hands of philosophers . . .".

Book V is devoted to astronomy during the Roman Imperial Period and Late Antiquity (that is, down to the seventh century AD). Book VI (pp1,061-1,456) contains Appendices, a Subject Index (including proper names), Bibliographical Abbreviations and so on, a short Greek glossary, and nearly 250 pages of diagrams and other figures, often as many as six on a page.

There is no doubt that here at last we have an up-to-date definitive textbook of ancient mathematical astronomy. I have so far noticed only one minor omission, and it may be deliberate. It concerns Thales, the sole reference to whom (p604) is to discredit his alleged prediction of the solar eclipse of 28 May 584 BC. Twenty years ago Professor A. Wasserstein contributed a paper to the *Journal of Hellenic Studies* (114-116, LXXV,

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Star-studded journey

Paul Davies

The Dark Night Sky: A Personal Adventure in Cosmology. By Donald B. Clayton. Pp. xii+206. (Quadrangle/New York Times: New York, 1975.) \$9.95.

I HAVE never read an astronomy book quite like this one. The author, who is an American astrophysicist at Rice University in Texas and an internationally respected scientist, has managed to weave a remarkable synthesis of personal narrative and descriptive astronomy into the same text. Not for Professor Clayton the dry old facts, figures and theories of conventional introductory astronomy books. Instead, reader discovers the motions of the Earth with the youthful Clayton delivering newspapers in Dallas. The mysteries of Stonehenge are unlocked as we accompany him in later years on a "sudden impulse" visit. Olbers' paradox unfolds from a detective story, as the author describes attempts to track down the family home of the eighteenth century astronomer de Chéseaux.

Much of this unusual book is taken up with the author's own research interest in stellar nucleosynthesis. Famous personalities like Fred Hoyle, William Fowler, Margaret and Geoffrey Burbidge appear as real people

(continued from previous page)

1955) on *Thales' Determination of the Diameters of the Sun and Moon*, followed soon afterwards by a further short note on the same subject, in which he argued that these determinations (ascribed to Thales by Diogenes Laertius) may have been obtained by angular measurement and not "as is generally supposed by time measurement". One other point concerns Neugebauer's conclusion (p643) that, in view of the highly inaccurate numerical values given by Aristarchus in his famous treatise *On the Sizes and Distances of the Sun and Moon*, this work should be regarded as "a purely mathematical exercise which has little to do with practical astronomy". I remember hearing a lecture by Professor Wasserstein ten or more years ago, in a colloquium at Imperial College, in which he made the same point.

The printing and presentation of all three volumes are up to the high standard we expect from Springer. Alas, not the least astronomical feature of this publication is its price. □

rather than mere names associated with pieces of work. To those, like myself, who worked in the early 1970s alongside Clayton and his friends at the Institute of Theoretical Astronomy in Cambridge, much of the account is nostalgic. In particular, the author's close friendship with Fred Hoyle (who has written a short foreword) comes through clearly—one whole chapter is devoted to their climbing exploits together in Scotland.

There is in fact much good science buried away in the text, but always interspersed with comment, narrative and a little Texas philosophy. The theory of relativity and some nuclear physics emerge from stories about Einstein and Rutherford, always embarked on by some personal incident. Emerging from the story is the unmistakable stamp of a romantic, helping to dispel the tired old idea of the cold, calculating scientist. The reader shares in the personal excitement which has changed the author's life. As we follow his daily experiences, science jumps out from everywhere: "Every aspect of nature" he explains "holds connections to the rest of the universe in my own mind . . . This sense of unity and knowledge has transformed my life from something mundane to something exciting".

Whether the reader wishes to learn astronomy by guided discovery, or to enjoy engaging stories of colourful personalities, Professor Clayton's book will provide an entirely welcome new perspective into the mysteries of astronomy and astronomers. □

Flavour of excitement in modern astronomy

J. E. Beckman

Astronomy and Cosmology: a Modern Course. By Fred Hoyle. Pp. xiv+711+23 plates. (Freeman: San Francisco, 1975.) \$15.95.

ASTRONOMY has been a shining beacon in the subdued world of physical science during the last decade. Whereas research funding for most branches of physics and chemistry has been in the doldrums, money for astronomical research has been expanding in real terms. The opening up of new ranges of the spectrum by new techniques and by the use of satellites, has meant new discoveries. One needs only to run through a familiar list—radio galaxies, supernova remnants, quasars, pulsars and black holes—and to realise how many of these have become almost

household terms, to understand why students have been turning towards astronomy and astrophysics as rapidly as they have turned away from some other branches of science. As a cultural subject, astronomy has further attractions for students and teachers. The history of the subject, the oldest recorded branch of natural science, subsumes a series of revolutions in the way man has viewed his place in the world. Even today the cosmological questions about the origin, the future and macrostructure of the universe exercise a unique fascination for both laymen and professionals.

Against this background Fred Hoyle has written a textbook aimed at first-year undergraduates. In US terminology it is a college rather than university text. A rapid glance through the book gives a most favourable impression. The author has made full use of the excellent illustrative facilities of his publisher, and their *Scientific American* style of didactic drawing. The book is well provided with photographs, mainly from the Hale Observatories, of nebulae, galaxies, and spectra. Above all the beautiful typography and layout go well with the leisurely presentation, in whose 700 pages the author has felt free to develop his favourite topics at considerable length.

One necessary reason for this spread is the deliberate abstinence from formal mathematics other than simple algebra and the use of logarithms, throughout the book, and the relegation of most algebraic development to chapter appendices. Although this had led to many elegant verbal explanations of topics as diverse as star formation, the quantum theory of spectra, and Kepler's laws, the result is to weaken the presentation. A perceptive student's feeling of inferiority before the mathematics will be increased; worse, a less able student will not realise the pervasive need for mathematical method in linking the concepts presented.

Nevertheless, the concepts are well presented and physical insight is demanded of anyone working through the interesting sets of problems, whose main virtue is to foster a feeling for orders of magnitude. The choice of material is a useful balance between classical introductory astronomy, and such modern topics as plate tectonics, and the physics of the 'big bang'. The author's occasional sallies into idiosyncrasy are carefully prefaced by "it is my belief" and like caveats.

I find the book excellent value at \$15.95, and although not entirely suited to a British first-year undergraduate course, as a gift to educate and interest a sixteen- to eighteen-year-old it is almost ideal. It does convey the flavour of excitement in modern astronomy. □

Mysteries of the angiosperm

W. G. Chaloner

Palaeobiology of Angiosperm Origins: Problems of Mesozoic Seed-Plant Evolution. (Cambridge Earth Science Series.) By Norman F. Hughes. Pp. vii+242. (Cambridge University: Cambridge, London and New York, January 1976.) £7.80.

THE past decade has seen a renewed surge of interest in the evolutionary origin and diversification of the angiosperms. Four major books (Hutchinson 1969, Taktajan 1969, Sporne 1974 and Stebbins 1974) have dealt with the problem, placing varying degrees of emphasis on comparative studies of living forms, with a proportionate lack of emphasis on the fossil record. This approach takes its most extreme form in Stebbins' assertion that "the fossil record of angiosperms can be more misleading than enlightening as a means of interpreting the major trends of their evolution". Hughes offers an encouraging refutation of Stebbins' morbid view. His book is a palaeontologist's unrepentantly partisan survey of the fossil record of angiosperms, and of the reputedly relevant gymnosperms of the past 200 Myr. He has as little time for comparative studies of living forms as Stebbins has for fossils.

Two important facts emerge from his thorough and well-illustrated analysis. First, there is no acceptable evidence of angiosperms before the early Cretaceous. Second, all those characteristic attributes of angiosperms which have a sporting chance of surviving in a fossil (wood with vessels, dicot-type leaves, multiaperturate tectate pollen and seeds enclosed in a fruit) appear within the timespan of one geological stage, during or close to the Aptian (late early Cretaceous). A vital contribution here has been the palynological evidence, repeated synchronously at widely separated sites, showing the sequential change from monaperturate to multiaperturate pollen types.

Hughes places great emphasis on the data-handling aspect of the angiosperm evolution problem. He is rightly concerned that a question-begging nomenclature should not conceal the inadequacies of our fossil record. But in some instances this is carried to alarming lengths. He worries over the application of 'dicot' to Cretaceous woods

or leaves, because the number of cotyledons is not directly evident in the fossils (he prefers the label 'dubiocot'). One wonders whether as a palaeontologist, he objects to attributing Cretaceous skeletal material to the mammals, since that designation is equally based on an attribute so singularly lacking in the fossil record?

The author offers us no snap answer to the mystery of angiosperm origins. But he has faith that the fossil record, properly documented, will eventually yield an answer. He hints that he believes the angiosperms to be polyphyletic, and that most of the major Mesozoic gymnosperm groups are under suspicion of duplicity. While we are awaiting that answer, this book is a most readable source of well-ordered factual information, and a strong goad to a renewed assault on one of the most rewardingly unsolved problems of biology. □

Jurassic-Cretaceous geology in Russia

John W. Neale

The Jurassic-Cretaceous Boundary and the Berriasian Stage in the Boreal Realm. Edited by V. N. Saks. Pp. vi+345+46 plates. (Israel Program for Scientific Translations: Jerusalem; Wiley: Chichester, September 1975.) £17.50.

ONE welcomes the translation of this impressive work which starts with a historical review of classification about the Jurassic-Cretaceous boundary, followed by detailed descriptions of the lithology, faunal succession, ethology and taphonomy of each relevant section in the Boreal Realm of the USSR. Western European developments are covered, although some up-dating is necessary in the light of recent publications, and correlation between the various areas is suggested. It is refreshing to find oneself in accord with this authoritative team of Russian workers in their reservations and strictures on the use of the term 'Ryazanian' and their preference for 'Berriasian'.

An extended review of the ammonites, belemnites, bivalves, brachiopods and foraminifera, read in conjunction with the 46 plates at the back, provides a fascinating insight into the Russian faunas. Although individual specialists may take issue with some of the generic or trivial assignments, this part provides an invaluable

guide to current Soviet thought on the interpretation of these groups. Both here and elsewhere a considerable number of new fossil species has been described by the editor/author whom the Russians transliterate as 'Sachs', so that the use of this form is obligatory in the attribution of these taxa and is correctly used in the translation. It is a pity that the translators feel obliged to follow western precedent and use 'Saks' on the cover and elsewhere in the running text. That 'Sachs' and 'Saks' are one and the same is a potential source of confusion, particularly to the non-specialist.

Otherwise, the translation is good, the occasional infelicities only serving to suggest the flavour of the original. Incidentally, both original and translation show a curious anomaly—in table 24, in which the *Bojarkia mensezhnikovi* zone is placed in the Valanginian in contrast to its firm placing in the Berriasian elsewhere. Interesting chapters on palaeogeography (although the maps take no account of continental drift and are rather small in the translation) and on palaeogeographic zoning, close the book. This handsome, if somewhat expensive, volume is beautifully produced and besides being an exciting mine of information to dip into, will remain an indispensable work of reference for many years to come.

Ecology and Evolution of Communities

Martin L. Cody and
Jared M. Diamond, editors

This book contains 18 major original papers, offering new models, methods and applications for analysing and interpreting a wide variety of empirical data. *Part I, The Evolution of Species Abundance and Diversity*; Richard Levins, Egbert G. Leigh, Jr, John W. MacArthur, Robert M. May, Michael L. Rosenzweig. *Part II, Competitive Strategies of Resource Allocation*; William M. Schaffer and Madhav D. Gadgil, Henry H. Hespenheide, Arthur M. Shapiro, Henry S. Horn. *Part III, Community Structure*; Martin L. Cody, James R. Karr and Frances C. James, Eric R. Pianka, James H. Brown, Jared M. Diamond, Ruth Patrick, Joseph H. Connell. *Part IV, Outlook*; G. Evelyn Hutchinson, Edward O. Wilson and Edwin O. Willis. *Belknap Press £17.70*

Anatomy of the Guinea Pig

Gale Cooper MD and
Alan L. Schiller MD

This is a thorough description of guinea pig anatomy illustrated with over 400 drawings by Dr Cooper, covering: external anatomy; the skeletal, muscular, cardiovascular, lymphatic, central nervous, peripheral nervous, respiratory, digestive, urogenital and endocrine systems; the ear; the eye and orbital contents. *Commonwealth Fund £20.00*

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Larger-than-life characters

M. J. S. Rudwick

The Dawnseekers: The First History of American Paleontology. By R. W. Howard. Pp. xiii+314. (Harcourt Brace Jovanovich: New York and London, October 1975.) £3.95.

THIS is a popular history of palaeontological research in the US, from the first great vertebrate finds in the eighteenth century to the full professionalisation of the science in the twentieth century. The style is journalistic, with racy narrative and fictional dialogue. The subject matter should be ideal for a good popularisation of the history of science, for many of the personalities involved were as colourful and as larger-than-life as the extinct animals that they discovered and reconstructed. The author claims that "*The Dawnseekers* was not meant to be a definitive history", but even the most modest popularisation should utilise the more accessible results of modern historical scholarship. The best parts of *The Dawnseekers* are those that describe the epic feats of Cope, Marsh and others in the newly-opened American West; but it is sad that the opportunity of setting this work reliably in its historical context has been so completely missed.

The story is still framed in the terms set (or at least, propagated) by that old warhorse of the 1890s, Andrew D. White, as a conflict between crusading scientists in shining armour and a benighted army of clerics and other obscurantists defending the sanctity of Ussher's date of 4004 BC. This kind of interpretation is inexcusable in the 1970s, when several readable books based on accurate historical research are easily available. To mention only one, the omission of which is all the more surprising because it rightly emphasised the importance of American discoveries, John Greene's *Death of Adam* (1959) placed those discoveries firmly in their historical context as part of a continuing debate on the implications of the history of life for human self-understanding.

Science needs good popularisations, and popularised history of science is an excellent way of communicating the historical dynamics and humanity of the scientific enterprise. Unfortunately the simplistic journalism of *The Dawnseekers* will do little to advance the lay understanding of science. □

Preparing *Brontosaurus gigas*, 1900



Courtesy American Museum Natural History

Geological clocks

A. Hallam

Growth Rhythms and the History of the Earth's Rotation. Edited by G. D. Rosenberg and S. K. Runcorn. Pp. xvi+559. (Wiley-Interscience: London and New York, September 1975.) £22.50.

In the 12 years that have elapsed since Wells published his pioneer paper on corals as geological clocks a large amount of research has been conducted on growth rhythms in living and fossil organisms in relation to solar and lunar periodicities. This book, the outcome of a NATO conference held in Newcastle-upon-Tyne, summarises this work, and contains in addition a number of interesting papers by geophysicists and astronomers on the Earth's rotation. These range from an analysis of eclipses from ancient historical records to more theoretical studies of the dynamics of the Sun-Earth-Moon system. Greatest interest attaches to the study of rhythmicity in fossil growth layers as the best hope of obtaining hard data on diurnal, monthly and annual cycles in the distant geological past. Although it is impossible to summarise adequately in a brief review the results of so many diverse contributions, it is apparent that early efforts in the field greatly underestimated the complexities in growth patterns that emerge from more detailed analysis, and that stromatolites come a poor third to bivalves and corals as research material. Some rhythms perceived in living organisms relate more to varying environmental conditions than to astronomical periodicities, and misinterpretation of fossils is likely to arise unless one has

access to large amounts of excellently preserved material with continuous growth records. There is also a need in the future for more objective measurements utilising, for instance, the optical densitometer. Nevertheless there still seems to be qualified support for a gradual slowing down of the earth's rotation throughout most of Phanerozoic time.

The editors are to be congratulated on a finely produced and splendid interdisciplinary effort. □

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Of crops and men

Joseph Hutchinson

Crops and Man. (Foundations for Modern Crop Science Series.) By Jack R. Harlan. Pp. xi+295. (American Society of Agronomy and Crop Society of America: Madison, Wisconsin, 1975.) \$11.25.

THIS is a good book. The expansion of studies of crop plant evolution in recent years has been so great that to produce a survey of the field in one volume is a daunting proposition. de Candolle and Vavilov provided respectively the roots and trunk of a tree that has now grown a crown almost too great to be encompassed by one man. Nevertheless, Jack Harlan has set out to do this. In his Preface he says: "The time has come for a third round of summary on what is known about crop origins".

Harlan has not only succeeded in accomplishing such a summary; he has contributed a most timely addition that substantially enlarges the field. de Candolle's interests were primarily botanical and geographical. Vavilov's were botanical, geographical and genetic. Harlan's are botanical, geographical, genetic and human. Although de Candolle was well aware of the human influences in the phenomena he studied, it was natural that for a long time the study of the origins of crops and of agricultural practice should be pursued separately. Their natural interrelation has been apparent in the past decade, and there are now numerous examples of the way in which biological and archaeological evidence together contribute to the elucidation of crop plant history. Harlan has taken these disciplines and fused them into a comprehensive account of the social as well as the biological changes that went into the evolutionary progression from hunting and gathering to a full agricultural economy.

The sponsors of the series of which this is the first volume, have sought to meet the needs of "upper level undergraduate college students". For students reading subjects in applied biology, archaeology, anthropology and geography this will be mandatory reading for a long time. It will also be invaluable for teachers who are developing 'A'-level Environmental Studies in schools.

This subject is my absorbing interest, and it would be surprising if I agreed with all of Harlan's views. I would add a South Asian Region to his list of

original crop regions, and would assign to it such crops as the Indian pulses (including pigeon pea), *Gossypium arboreum*, and some tetraploid and hexaploid wheats. I think he has overdrawn the protein shortage, and I do not think one can accept his statement (p253) that "it was not until World War II that significant increases in per hectare yields were achieved". There were at least two earlier periods in British agricultural history during which substantial advances in yield were made. But these are matters for debate, and it is a measure of the stimulus of the book that one constantly made a mental note of a new or unrealised aspect of the subject—that the hunter-gatherer lived in a golden age, for example—or of an interpretation one did not accept and would like to discuss with the author.

The book begins with a discussion of the way people lived before they farmed, how they began to farm (leaving open the question why they should want to), and our present ideas of the areas in which farming began. Then there are chapters on crops and weeds—which is which and why—and on the classification of cultivated plants. Genetic and evolutionary topics follow. Five chapters are devoted to regional studies, and finally there is one on recent spread much of which can be documented historically, and one of interesting and well controlled speculation on future prospects and possibilities.

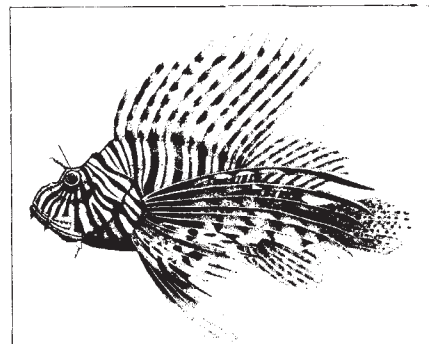
I enjoyed it. □

Air pollution

C. P. Whittingham

Responses of Plants to Air Pollution. (Physiological Ecology: a Series of Monographs, Texts and Treatises.) Edited by J. B. Mudd and T.T. Kozlowski. Pp. xii+383. (Academic: New York and London, November 1975.) \$29.50; £15.35.

THE study of the response of plants to aerial pollutants is relatively new. In our present state of ignorance it is important to differentiate between proven facts and generalisations which have become established only by frequent repetition. There is a need to pose pertinent questions which require experimental investigation and to formulate primary problems in analytical terms. Unfortunately this book does not attempt these tasks. It consists largely of literature reviews of current work and since all the authors are American most of the book relates to conditions in America and to work undertaken there.



Scorpion fish (*Pterois volitans*). Taken from *An Age of Fishes: The Development of the Most Successful Vertebrate*. By Joy O. I. Spoczynska. Illustrated by Melchior Spoczynski. Pp. 145. (David and Charles: Newton Abbott, London and Vancouver, February 1976.) £3.95.

Separate chapters discuss the influence of sulphur dioxide, ozone, fluorides and peroxyacyl nitrates, oxides of nitrogen, and particulates. Some chapters include more references than others and are correspondingly more valuable, but essentially all are concerned with reviewing the literature rather than discussing principles. Subsequent chapters deal with the response to mixtures of pollutants and the interaction of air pollutants with vegetation. There is little reference to the importance of the physiological status of the plant—for example, the influence of water stress or mineral deficiency in relation to plant response to pollutants—and there is only a limited reference to the importance of aspect in relation to pollution damage. One chapter is concerned with the interaction of pollutants with vegetation canopies; unfortunately there is little discussion relating the spread of pollutants to physical processes. Other chapters are concerned with the specific effects of pollutants in forests and in relation to agriculture. There is also a chapter on the influence of pollutants on plant structure and on the growth of lichens and bryophytes. In many chapters reference is made to the biochemical action of specific pollutants and perhaps it is premature to seek an account of the general interaction of pollutants with plant metabolism.

The book will be useful to those who require an introduction to the literature of plant response to pollutants. It does not provide a critical view of our present state of knowledge of the subject. It makes general statements without quoting evidence such as "yields of virtually all important crop plants have been greatly depressed by air pollution"; with certain exceptions it fails to emphasise the important facts which still need to be established in the face of the possible need to define air quality standards for future legislation. □

Ecology of rivers . . .

D. H. Mills

River Ecology. (Studies in Ecology, Vol. 2.) Edited by B. A. Whitton. Pp. x+725. (Blackwell Scientific: Oxford and London, 1975. Distributed in US by University of California Press.) £18.

River Ecology is the second volume in this internationally-based series concerned with ecology which aims at the production of scholarly integrations of established topics and critical assessments of newly-opened fields. This volume offers a comprehensive treatment of river ecology, and its prime aim is to provide a fundamental framework for the subject. Such an approach will be of value both to the more advanced student and to the research worker and river management officer who needs some guidance to the mass of literature which is now appearing about rivers. The 28 contributions to this book go a long way to fulfilling these aims and have certainly guaranteed that it is one of the few, although not the cheapest, books in English which covers so many aspects of river ecology.

Unavoidably the contributions vary in their quality and length, and their uneven size does not necessarily correspond to the importance of their subject. Some are disappointingly short, whereas others are surprisingly drawn-out. For example, only 31 pages are devoted to fish, whereas a similar number of pages deal with lotic periphyton. No-one would question the great importance of zonation in river ecology but the 63 pages devoted to this subject are more than twice the number of pages given to any of the other contributions except two.

In a book of this sort, where the size of the author's contribution is necessarily limited, it is not difficult to find omissions. The most glaring omissions occur in the chapter on Ecological Aspects of River Impoundment. This chapter tends to dwell almost entirely on the effects within the impoundment, and the downstream effects of impoundment on rivers are described in two pages. Only ten lines are devoted to the effects of hydroelectric installations on river flows and fish movement, and no mention is made of the effects of water transfer, either directly from river to river or indirectly through a regulating reservoir. This is a subject of paramount importance in the water

resources field in this country at the moment, and its omission is to be deplored.

In the chapter on River Zonation and Classification a number of photographs are included which depict the various zones, named after the dominant fish species present, in a number of river systems. A number of these do not correspond with the physical appearance of these same zones in other river systems. For instance, the example given of the minnow reach or grayling zone (Fig. 14.2a) is nothing like the grayling zone on the River Tweed or the River Clyde; and the minnow reach in some Welsh mountain streams would correspond almost to the head stream depicted in Fig. 14.1a. The author refers to the River Wye, at Newbridge-on-Wye, (Fig. 14.2b, and the front cover of dust jacket) as being an upper salmonid zone although grayling, dace and chub are abundant in this stretch of the river. I would not refer to the Wye being an upper salmonid zone until one reached a point upstream of Llangurig.

The book ends with an Appendix which gives a guide to Further Literature on Particular Rivers. There are a number of glaring omissions, however, which include the Teifi and Towy in Wales, the Bran, Conon, Don, Forss, Meig and Tay in Scotland and the Fraser, Margaree, Miramichi and Pollet in Canada.

This is, however, a most valuable work on river ecology and I recommend it being purchased by all appropriate university departments and research institutions, and by more affluent research workers. □

. . . and animal populations

M. E. Solomon

Animal Population Ecology. By J. P. Dempster. Pp. x+155. (Academic: London and New York, August 1975.) £3.80; \$10.00.

THIS thin, hard-backed volume provides good, if mostly brief, coverage of its subject, dealing with 'basic concepts', competition, social behaviour, qualitative changes, natural enemies, weather, and examples of field studies, and concluding with population theories and applied population ecology. The comparative account of selected field studies is a major and valuable feature. For each of them, life

tables are presented and analysed by the Varley and Gradwell method for key factors, to diagnose where, if at all, in the life cycle there occurs a mortality that is the chief component of the fluctuations in numbers over successive generations. The book also attempts to diagnose which process is responsible for the limitation of numbers. In four of the six examples, this density dependent process seems to be intraspecific competition, for food in the cinnabar moth, wood pigeon, and pine looper in its outbreak areas, for settlement sites in the edible cockle, or for territories in the tawny owl. Three other field studies, on larch tortrix, red grouse and rock ptarmigan, are considered in connection with the problem of how periodic cycles of these species are engendered.

The author's grasp of the theory of population dynamics seems to falter in places. The intrinsic rate of increase (r) equals births minus deaths (p. 4) only in the infinitesimal interval of the calculus equation describing increase; over a finite period the relationship is not so simple. It is not true that k -factor analysis necessarily enables us to recognise "any density dependent relationships which exist" (p. 15). The statement (p. 140) that "the maximum sustainable yield.....in the logistic model.....is $K/2$ ", can hardly be correct, since in this context yield is a rate, whereas $K/2$ is a quantity.

The exposition is occasionally faulty. On page 17 the explanation of k -values is confused by the introduction of an index of mortality that is insufficiently explained by the footnote. In Table XVII the values are divided by 10^6 and so on, not multiplied as indicated above the columns. The phrase "sufficiently density-dependent to prevent extinction" (p. 105) may lead students to suppose that a density-dependent influence at low density can avert population extinction in some positive way (whereas the most it can do is to cease its adverse action). On the verbal level, it is disconcerting to find Pimentel, one of the theorists whose views are examined, misspelled in both text and reference list. 'Epidemic' should not be treated as a synonym of 'epizootic' (p. 43). Hassell's term is 'aggregative', not 'aggregate' response (p. 49).

In spite of these blemishes, however, this is a valuable book that should appeal to university students and research workers. It has an index and an up-to-date reference list of some 200 items. Its scope overlaps that of the earlier book by Varley, Gradwell and Hassell (*Insect Population Ecology*, Blackwell, Oxford, 1973), which goes more deeply into population dynamics and the relevant methods of analysis, but is confined to insects. □

On trial

Griffith Edwards

Brain, Behaviour and Drugs. By David M. Warburton. Pp. x+280. (Wiley: London and New York, December, 1975.) £6.95; \$15.30.

TEXTBOOKS on psychopharmacology are these days appearing thick and fast and there might be a case for a 'Which' report. The present book is certainly a good buy, but as in every consumer's report there are a few things to niggle about. The particular attraction of this subject is that it enables authors to put on it an individual stamp, and here the stamp is characterised by authority and enthusiasm.

Dr Warburton first discusses the chemistry of synaptic transmission, and the effect of drugs on transmission. An interesting chapter then focuses on control of homeostatic motivation, with particular reference to water balance and food intake. A chapter on the biochemical basis of mood competently covers essential ground, and the section on control of attention gives a careful account of a difficult area. There is a good section on schizophrenia but affective illness does not get similar treatment, and intelligence and learning are also covered.

It is in the later sections that there is occasion for a few criticisms. The review on the influence of alcohol on the EEG is not up to date. The section on the biochemical basis of drug dependence, although basically very well handled, shows some flaws. For instance, the statistic is quoted that 22.5% of the psychiatric admissions in Brazil, India, Morocco and Nigeria were between 1957 and 1964 as a result of an acute cannabis reaction—a statement so bizarre as usefully to be put before students to test their critical faculties, but not to be coldly retailed as fact. To say that personality changes consequent on cannabis use "seem to be irreversible", runs some leagues ahead of the evidence, and the cannabis literature is in general not sufficiently well treated.

The chapter on anxiety and stress is also a little bit disappointing. The concept of anxiety and the concept of stress could both have done with greater elucidation: the student might expect more about the measurement of psychophysiological correlates of anxiety. The discussion on the clinical application of anti-anxiety drugs on human beings errs on the side of optimism—not every psychiatrist would agree that "the debilitating

effects of pathological anxiety have been reduced to a remarkable extent by a number of psychoactive drugs."

The usual difficulty with the teaching text on this subject lies in effectively making the linkage between the psycho- and the -pharmacology. This book succeeds more than most. The real way to test such a new product must of course be to try it out live on a panel of consumers, and here that means seeing how the book actually goes as a teaching text. This book certainly deserves that kind of trial. □

Neural organisation

H. F. Bradford

The Synaptic Organisation of the Brain: an Introduction. By Gordon M. Shepherd. Pp.xi+364. (Oxford University: London and New York, 1975.) £3.80.

WE have not yet reached the position where a definitive account of the synaptic organisation of the brain can be given, but Professor Shepherd provides us with a useful current appraisal of our degree of success in the drive towards that objective. He begins with a brief introduction to the morphology of synaptic and related structures and of neural connexions, and proceeds to expound the biophysical mechanisms which probably underlie resting transmembrane potentials and their displaced versions, the action and synaptic potentials. This is done in an explanatory way rather than with a vigorous mathematical approach. This leads on to an analysis of dendritic organisation in relation to passive spread of potential change due to synaptic activity, and the work of Wilfred Rall in this area. The second and most dominant part of the book deals with neural organisation in the ventral horn of the spinal cord, the olfactory bulb and cortex, the retina, the cerebellum, the thalamus, the hippocampus and the neocortex. The text is based on an advanced seminar course given by the author in recent years at the Institute of Neurological Sciences at the University of Pennsylvania, and its aim is to isolate and propose principles of synaptic organisation in these regions for the consideration of advanced students and research workers in neurophysiology. Neuropharmacological and developmental aspects of the subject are purposely restricted to keep the length of the book within reasonable bounds.

I found the flow and content of the book interesting and engaging as well as informative, and it is well linked to the literature of the subject with

copious references (a large proportion dating from 1970 onwards). The absence of the more recent developments in our knowledge of catecholamine and indoleamine-operated pathways in the mammalian brain does detract from the success of the book as a description of synaptic organisation, but one appreciates the reasons for their absence. The accounts of the neurophysiological mechanisms of the various brain regions are clearly explained and well illustrated, and the rather conversational style of the text, together with the author's determination to present complex mechanisms as interplay of simpler more universal processes, adds to the ease of its reading. Each regional account deals with the range of neurone types present and the major types of synaptic connections existing between them. This is followed by an account of organisation in terms of circuit diagrams and the special features of that region in this respect. This is accompanied by a discussion of specialised dendritic properties crucial to the synaptic interactions. There are a large number of books in this area appearing at present, but this should prove to be among the most popular. By modern standards its price is attractive for its size and content, and in this respect too it should compete successfully on the bookstalls.

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Tranquillisers no answer to schizophrenia

Malcolm Lader

Drugs, Madness and the Brain. By Solomon H. Snyder. Pp. 287. (Hart-David, MacGibbon: London, 1975.) £6.

It would be a macabre task to draw up a league table of the most terrible diseases which can afflict mankind. In such a list, schizophrenia would be very near the top as this illness disrupts and destroys normal mental functioning. It is common, one in a hundred adults succumbing. The outcome is uncertain, some recovering unscarred, others with residual symptoms and strange behaviour, and an appreciable unfortunate group who occupy the majority of the 'chronic beds' in health services throughout the world. These patients exist in institutions ranging from true mental hospitals, with rehabilitation and return to the community as the aim, through lunatic asylums, where the patients are cared for to a greater or lesser extent, to 'bins', a pejorative but accurate slang term.

New hope dawned for schizophrenic patients, their families and medical attendants with the advent of the major tranquillisers 20 years ago, coinciding with more liberal attitudes towards the mentally ill. The numbers of patients in hospital declined although the number of admissions rose.

In this book, Solomon Snyder, a psychiatrist-cum-pharmacologist, sets out for the layman recent research into the biology and treatment of schizophrenia. In part I he outlines concepts of insanity, the treatments for schizophrenia and the effects of the psychedelic drugs like mescaline and lysergic acid diethylamide (LSD) pointing out that the psychoses which these drugs induce differ in several crucial respects from schizophrenia. Part II provides a superb non-technical description of the many mental aberrations and behavioural peculiarities of the schizophrenic patient. Finally, Snyder describes the psychosis induced by repeated high doses of amphetamine and shows how similar the mental state is to that of paranoid schizophrenia.

One tantalising question remains unanswered. What is the clinical type of

action of the major tranquillisers such as chlorpromazine? Are these drugs anti-psychotic, that is, lessening psychotic symptoms whatever the causation, or are they truly anti-schizophrenic, influencing the schizophrenic disease process itself? Although Snyder heads one chapter "Anti-schizophrenic Drugs", he is at pains to point out the contradictions implicit in this rubric. For example, schizophrenic patients are not all cured and some sceptics have suggested that the major tranquillisers are merely 'chemical strait-jackets'.

The most relevant pharmacological effect of the major tranquillisers has recently become clearer. All these drugs have the common property of attenuating the action of the neurotransmitter dopamine by competitive, postsynaptic blockade. This explains extrapyramidal side-effects like Parkinsonism and some hormonal effects of the drug. It is tempting to postulate that schizophrenia involves dopaminergic overactivity in the brain but Snyder points out the dangers in such

a facile extrapolation. This area of pharmacology is growing rapidly and should uncover the biochemical and physiological mechanisms associated with psychotic behaviour in general and (dare one hope) schizophrenia in particular.

The book is well written in non-technical language. It is entertaining, vigorous, optimistic in outlook but balanced in opinion. It can be heartily recommended to any intelligent layman who wants to keep abreast of research development in a major area of health concern. It should also be read by medical and scientific professionals from medical and psychology students to researchers involved in the field. And our administrators and politicians involved in the provision of health care might also take time to learn about schizophrenia—this dreadful life-long mental condition which afflicts so many thousands of patients vegetating in institutions or existing precariously in the community. Major tranquillisers are still not a substitute for adequate health services. □

Pervasive role for acetylcholine

T. J. Crow

Central Cholinergic Systems and Behaviour. By P. V. De Feudis. Pp. x+422. (Academic: London and New York, 1975.) £6; \$15.75.

At a conservative estimate, established chemical transmitters in the central nervous system must now number at least seven, even excluding polypeptides which act as hypophyseal releasing factors, the recently discovered endogenous morphine-like compound, and the elusive substance P. Why so many substances should be needed to carry messages from nerve cell to nerve cell is a major unsolved question. The classical concept of postsynaptic excitations and inhibitions suggests a requirement for two, and the present surfeit might be thought to support the view that major varieties of action at the post synaptic cell have yet to be described.

Among these agents acetylcholine has a particular place, as the first such substance convincingly to be demonstrated to have a neurohumoral role, and by reason of the detailed knowledge we have of its release and action in the periphery. Yet its functions in the brain might be thought recently to have been neglected by comparison, for example, with the monoamines.

This monograph by de Feudis contributes toward redressing the balance and reviews in detail the information which suggests that acetylcholine undoubtedly has a pervasive role. Although much of the subject matter concerns experimental findings at the electrophysiological and cellular level the book is organised in terms of functional systems and behaviour. The author does not flinch from plunging the reader into a first chapter on cholinergic mechanisms and consciousness, and follows this with sections on motor systems, and the role of acetylcholine in autonomic and endocrine control, and in motivated behaviours, emotion, learning and memory. The reference in the title to behaviour is therefore justified and the book will be of particular interest to physiological psychologists. De Feudis has been unusually successful in his attempt to bring together a variety of relevant studies at the biochemical and cellular level and at the level of gross structure and function.

A text organised around a single neurotransmitter substance invites questions concerning the lead which information concerning this substance might give us to the functional organisation of neurotransmitter mechanisms in general. When does a pathway need to use acetylcholine as a neurotransmitter? Anatomical precision is crucial here, and as de Feudis makes clear, in spite of the development of the cholinesterase technique, there are many limitations in our present knowledge of the anatomical identity of cholinergic neurones. □

Adrenergic transmission

A. D. Smith

Adrenergic Neurones: Their Organisation, Function and Development in the Peripheral Nervous System. By Geoffrey Burnstock and Marcello Costa. Pp. xi+225. (Chapman and Hall: London, August 1975.) £8.50.

THIS comprehensive little book presents a summary of our knowledge of the peripheral adrenergic nervous system. It serves as an excellent entry into the literature, for the authors have quoted nearly 1,200 references which occupy 80 pages. Since only 136 pages remain for the text, it is inevitable that much of the writing is very descriptive. This is particularly so in the first two-thirds of the book, which gives a rather superficial account of the general organisation and structure of adrenergic nerves, the biosynthesis and metabolism of noradrenaline, and the basic features of neurotransmission. The remainder of the text, starting with a discussion about adrenergic transmission, continuing with a chapter reviewing the growth and degeneration of adrenergic neurones and ending with a general discussion, is much more original and contains some important new ideas.

It is hardly surprising that, in their wish to be both brief and comprehensive, the authors have occasionally oversimplified and have tended to make dogmatic statements. The reader is likely to be rather confused by the account of ganglionic transmission where it is stated that inhibitory muscarinic cholinergic receptors occur on adrenergic neurones (p14) although elsewhere the muscarinic inhibitory effect is said to be mediated by interneurons that release a catecholamine. No mention is made of the excitatory muscarinic cholinergic receptors in ganglia; perhaps it would have been wiser to admit that the events in the ganglion are complex and not fully understood. Likewise, it would have been better to say that we know nothing about the electrogenesis involved in the muscarinic action of acetylcholine on nerve terminals, which leads to inhibition of the release of noradrenaline evoked by orthodromic stimulation, rather than to imply that it is due to hyperpolarisation of the membrane. I assume that the authors supposed that hyperpolarisation of the membrane in the varicosities might block the invasion of the varicosities by the action potential; however, if the action

potential is in fact conducted in the varicosities, then hyperpolarisation would increase its amplitude and so increase the amount of transmitter released.

The authors claim that the re-use of synaptic vesicles in nerve endings is not compatible with the idea that noradrenaline is released, together with dopamine- β -hydroxylase, from the vesicles by exocytosis (p34). I can see no inconsistency since after a vesicle, that has newly arrived in the terminal following transport from the cell body, has released its noradrenaline and soluble dopamine- β -hydroxylase, the



"Freckie (the otter) is displeased". Taken from *The River People*. By Philip Wayre. Pp. 180. (Collins and Harvill: London, 1976.)

vesicle membrane can probably be retrieved from the plasma membrane to form a new type of vesicle. The new vesicle is perfectly functional since it contains the (non-releasable) membrane-bound dopamine- β -hydroxylase and can store noradrenaline by means of the pump in its membrane. Such a re-use of the vesicle membrane provides an answer to the authors' problem that, if each vesicle is only used once, then the supply of vesicles by axoplasmic transport from the cell body to the terminal is apparently not adequate to maintain transmission (p91).

One of the best features of the book is the discussion of neuroeffector transmission. Here the authors illustrate the key importance of the size of the gap between the terminal varicosity of a neurone and the effector cell. In some tissues (for example, vas deferens, nictitating membrane, sphincter papillae) the gap is about 20 nm, whereas in blood vessels the minimum gap is larger and is also more variable (80-400 nm). Functionally, this range of distances means that those tissues

in which the gap is small respond to nerve stimulation more quickly and at lower frequencies of stimulation than do tissues in which the gap is greater. Many of the differences found by pharmacologists in the responses of the various adrenergically innervated tissues to drugs can now be accounted for in terms of the size of the neuromuscular gap. In tissues in which the gap is fairly wide, the peak concentration of noradrenaline at the receptors following nerve stimulation may be close to the threshold concentration. In these tissues, decreasing the amount of transmitter released, either by depleting the vesicle stores (with reserpine) or by interference with the release process (with bretylium), is likely to inhibit neurotransmission. In contrast, in tissues in which the gap is small these two drugs have little effect on transmission because the peak concentration of noradrenaline at the receptor may normally be more than 100 times the threshold concentration. Such a high peak concentration following nerve stimulation could also account for the lack of effect of α -blocking agents on neurotransmission in the vas deferens since the concentration of blocking drug would not be high enough to compete with the transmitter. Finally, the ability of drugs like cocaine to potentiate neurotransmission in some tissues but not in others is most easily explained by the different size of the neuromuscular gap.

The lack of uniformity in the organisation of adrenergic neuroeffector systems has led the authors to propose a fascinating new hypothesis that the terminal network of an adrenergic neurone not only permits direct control of the smooth muscle by the nervous system but also acts in those tissues that are densely innervated, to protect the tissue from the influence of circulating catecholamines. This protective function is the result of the ability of the adrenergic neurone to sequester catecholamines that are in the extracellular space; the denser the innervation, the more effectively are catecholamines removed. The authors point out that densely innervated organs such as the vas deferens and iris perform functions that are quite unrelated to those of the adrenal medulla. This hypothesis represents the first serious challenge to the classical views of Cannon, who saw the sympathetic nerves and the adrenal medulla as, functionally, one physiological system. This book is thus doubly welcome for it not only serves as a useful and comprehensive introduction to the field but also provokes physiologists and pharmacologists to take a fresh look at adrenergic neurotransmission. □

Brain power for astronomy

W. H. McCrea

The Place of Astronomy in the Ancient World. (A Joint Symposium of the Royal Society and the British Academy.) Edited by F. R. Hodson. Pp. 276+25 Plates. (Oxford University: London, 1975) (Published for the British Academy.) £13.

'ASTRONOMY in ancient literate societies' (Part I) starts with a useful introduction to needed astronomical concepts and closes with an account of two uses of ancient astronomy, both contributions by R. R. Newton. These ensure maximum comprehensibility of the work and appreciation of some of its objects, the two chosen by Newton being the study of the non-gravitational acceleration of the Earth and Moon, and the study of chronology, particularly from ancient eclipse records. The intervening contributions are on Babylonian mathematical astronomy, Babylonian observational astronomy, ancient Egyptian astronomy, astronomy in ancient and medieval China, and Mayan astronomy in central America. For most readers the striking general features must surely be the widespread character of astronomy in the ancient world and the inference that scientific astronomy in Greece, India, Islam and western countries was so much derived from Assyrian and Babylonian astronomy. Indeed, there is no chapter on Greek astronomy as such; presumably the characteristic Greek contribution is to be regarded as one of interpretation rather than discovery.

Part II is on 'Ancient astronomy: unwritten evidence' with contributions on 'Neolithic science and technology', 'Polynesian and Micronesian astronomy', 'Prehistoric monuments in western Europe', 'Astronomical alignments in Britain, Egypt and Peru', 'Archeological tests on supposed prehistoric astronomical sites in Scotland'. These deal with the most recent sort of study of ancient astronomy and that which has attracted most popular attention. There follows a chapter by H. H. Lamb on 'Climate, vegetation and forest limits in early civilised times' and one by D. G. Kendall on 'Hunting quanta'. These two are highly relevant in topic and they show the extraordinary range of science that has to be brought to bear on the problems concerned. The investigation as to whether the dimensions of megalithic sites reveal the use of a quantum

(5.44 feet, 1.66 m) demands novel mathematical statistics of subtle ingenuity (but it seems not to have been tested by re-discovering the foot or the metre!). The book includes brief reports of some informed discussion at the meeting.

Within the allotted space, the book provides surely the best possible accounts of its topics. It is a feast of learning and scholarship, strongly spiced with wit and wisdom. It is thought-provoking in innumerable ways; some of these are noted in the concluding remarks by D. G. King-Hele and by S. Piggott from the sides of the physical scientists and the archaeologists. Naturally one of the most obvious features of some of the unwritten evidence is its uncertainty. On the whole, readers may be inclined to agree with King-Hele that some features of prehistoric sites did indeed mark extreme rising and setting points of the Sun and Moon, and the like, whereas a connection with eclipse prediction seems less likely. As regards the alignments, E. W. MacKie in his contribution remarks explicitly on the difficulties of the subject, but he then asserts: "The plausibility or otherwise of these alignments is something all can assess by visiting the sites". Actually a party of Cambridge undergraduates did subsequently visit some of the sites and came away rather dubious about the alignments (M. E. Bailey *et al.*, *Nature*, **253**, 431, 1975). But MacKie's assertion is surprising because the problem tackled by Kendall is the most straightforward in the subject and yet all his powerful statistical apparatus is unable to produce a definite conclusion from the available evidence. All the other inferences about the sites seem to be *a fortiori* quite indefinite—but fascinating and deserving of the most thorough-going investigation.

The brain power of even the most ancient of the peoples concerned—as King-Hele says—cannot have been appreciably different from that of our contemporaries. This raises all manner of questions about the progress of knowledge and understanding of the astronomical world. Our knowledge is more than that of the ancients, but is our ignorance as great as theirs? Does almost constant brain power produce accelerated growth of technique and accompanying increase in knowledge? Or do developments occur in fits and starts with peaks of knowledge and understanding and great gaps between the peaks? Is our knowledge placed on record in such a way that people 3,000 years hence will be aware of what we know much better than we are of what people knew 3,000 years ago? This book will provide material for endless debates. □

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Worm's eye view

Neil A. Croll

The Biology of Free-Living Nematodes. By Warwick L. Nicholas. Pp. viii + 219. (Clarendon: Oxford; Oxford University: London, August 1975.) £8.25.

THE study of nematodes, perhaps more than with any other group of animals, is divided by the artificial barriers of professional groupings, journals and institutions. Too little communication exists between 'helminthologists', 'plant nematologists' and 'invertebrate biologists'. Dr Nicholas's book brings establishment to another faction, the free-living nematodes. As such it further frustrates the attempts of those who would study nematodes as a single biological grouping; it does, however, recognise the important role of the new nematologists who specialise in 'free-living' forms. 'Free-living' is a difficult notion; essentially it describes what is left when the parasitic species are taken away. Such a definition has hazy edges: parasites often have 'free-living' larvae, fungal feeders are free-living, but species which feed on higher plants are parasitic. Dr Nicholas wisely does not attempt strict definitions and emphasises soil, fresh water and marine forms.

As a series of essays some chapters can be recognised as those in which Dr Nicholas has had direct personal experience—such as 'Culture and Nutrition', Cytogenetics and Development' and 'Biochemistry'—and these achieve a high level of comprehension and accuracy. The chapter on 'Ecology' is a clear and thoughtful assemblage of a very heterogeneous subject area, and is the best short review of the subject currently available. One of the most attractive features of the book is that marine nematodes are not neglected. Furthermore, they are set apart from other free-living species on basic biological criteria but at the right distance.

In the first chapter on Morphology and Structure, much of the information is drawn from nematodes other than free-living forms. This has introduced some patchiness and has resulted in such important sections as those on the sense organs, excretory system and cryptobiosis being weaker than the other more restricted parts of the book. References are quoted but they are not necessarily the most relevant; they do not always demonstrate a synthesis made by the author before their selection. The book is eminently readable and the few typographical errors and

mis-spelled names are more distracting than misleading. The book is informative rather than scholarly. Dr Nicholas has chosen a chatty style which narrates the subject rather than one that provokes the reader to think beyond these pages. Typical of this are his useful chapters on taxonomy and methods of handling and culturing.

There are over 1000 references but only 20 are quoted after 1971 and none after 1973. This has resulted in certain topics, in which rapid growth has occurred, already being dated; it is, however, more contemporary than any other competing text.

Nematodes are being widely used as model metazoans by geneticists, behaviourists, gerontologists, and developmental biologists. This is the best book available to give these researchers and their students in allied disciplines a 'worm's-eye view' of the nematodes. □

Fiddler crabs

R. G. Hartnoll

Fiddler Crabs of the World: Oryzodidae—Genus Uca. By Jocelyn Crane. Pp. xxiii + 736. (50 plates.) (Princeton University: Princeton, New Jersey, November 1975.) £43.70.

WITH over seven hundred large pages, and weighing in at only a shade under 3 kg, this is a truly impressive book at first sight. It is a pleasure to report that this favourable opinion is reinforced by a study of the contents. This monograph covers all the known species of *Uca* in respect of taxonomy, functional morphology and behaviour. To a great extent it is based on many years of the author's own work in various regions of the world, and as a consequence the different topics are integrated to an unusual degree.

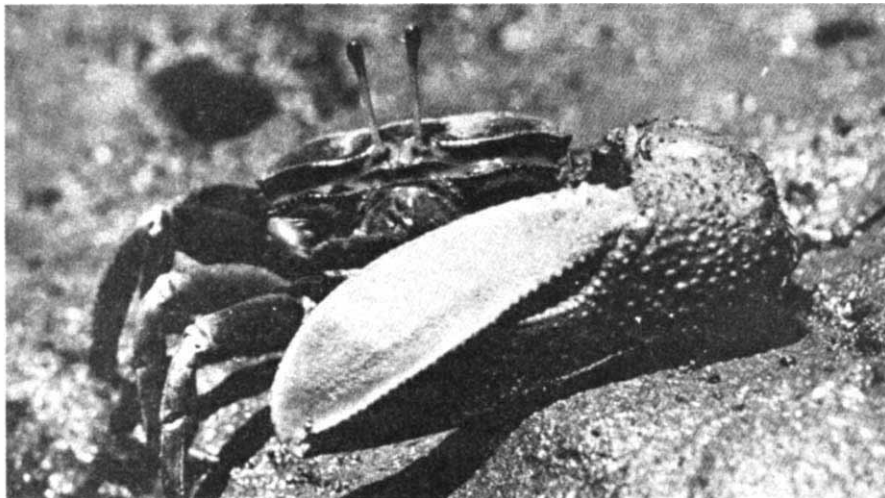
The first and larger part of the book is the systematic section within which

each of the 62 species of *Uca* is dealt with in turn. For each one there is a full morphological description together with an analysis of its differences from related species, followed by details of behaviour, distribution and type material. I was relieved to find that the genus *Uca* was not fragmented here, for although large it has a distinctiveness and homogeneity which render it an excellent example of the generic concept. Recently Bott (*Senckenberg. biol.*, 54, 315–325; 1975) has proposed the division of *Uca* into nine genera, but it now seems unlikely that this partition will be generally accepted. Dr Crane renders the genus manageable by introducing a new scheme of subgenera, and simplifies a number of taxonomic difficulties by a judicious use of subspecific categories. Her analysis of criteria for erecting subspecies is particularly interesting.

The second part of the book contains a series of chapters which collate and discuss, subject by subject, the information presented species by species in the first. There are excellent chapters on zoogeography, environmental relationships and functional morphology, but to me the most interesting are those on behaviour and evolution within the genus. Few large genera have been analysed in such detail with regard to these latter.

There are a number of appendices, of which the most useful are the keys to species in different geographical areas, and that providing practical advice on the observation, collection, transport and maintenance of these crabs. There are many high quality line drawings and photographs which amplify both the morphological and behavioural accounts. Overall this is a remarkably comprehensive review of a most interesting genus, and has been produced to a very high standard. It will be of great interest to carcinologists of a variety of disciplines, and it is only a pity that the economics of publishing must put it beyond the reach of most. □

Male fiddler crab (*Uca maracoani maracoani*), Trinidad



Biology of man . . .

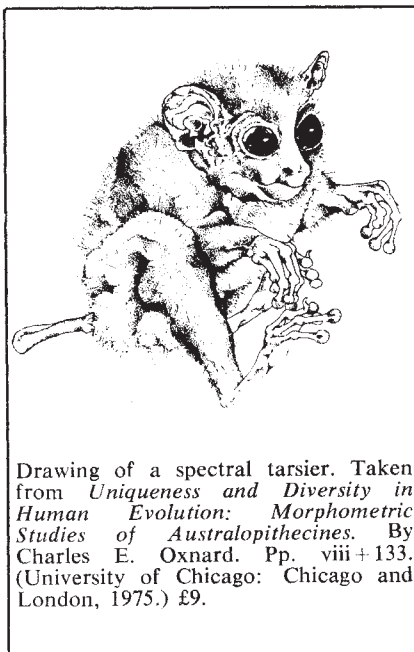
Cedric A. B. Smith

Human Variation and Natural Selection. (Symposia of the Society for the Study of Human Biology. Volume 13). Edited by D. F. Roberts. Pp. ix+209. (Taylor and Francis: London, 1975). £4.50.

THIS book consists of a selection of papers read at meetings of the Society for the Study of Human Biology about 15 years ago, and published in previous symposia about that time. In spite of the lapse of time since then these papers still have freshness and relevance. A considerable amount of detailed knowledge has accumulated in the topics dealt with in many of the papers since they were written; but the basic ideas have not changed very much, even though the emphasis may now be different.

The first topic of importance essentially concerns the factors shaping the relative frequencies of various inherited characters in a natural population. There are contributions on that by L. S. Penrose, A. R. G. Owen, C. A. Clarke, P. M. Sheppard and T. Dobzhansky. They explain three main ways of maintaining variability in a population, namely by heterozygote advantage, by mutation counter-balanced by natural selection, and by natural selection favouring different types in different habitats. There are three main ways of introducing genetic variability for a limited time: genetic drift, and the response to changing natural selection, either because of a temporary change (for example, an epidemic) or a permanent shift in environment. Fifteen years ago we knew one clear case of heterozygote advantage, the sickle-cell polymorphism in Africa, described by Allison in a later paper in this book, and a number of cases of balance between mutation and selection. Although theory has been developed much further, observational knowledge remains substantially as before.

Another group of papers, by A. E. Mourant, A. C. Allison, N. A. Barnicot and S. M. Gartler, examines various Mendelian characters which show polymorphism, such as blood groups, haemoglobins, haptoglobins, transferrins and amino acids. Except for certain polymorphisms, such as that of abnormal haemoglobins, which



Drawing of a spectral tarsier. Taken from *Uniqueness and Diversity in Human Evolution: Morphometric Studies of Australopithecines*. By Charles E. Oxnard. Pp. viii+133. (University of Chicago: Chicago and London, 1975.) £9.

seem to be related to the presence of malaria, these characters seem suitable for anthropological investigations designed to find which populations are most closely related. Later work has extended this list, especially as regards enzyme variability.

There are also papers by A. C. Stevenson on "Biological studies of small communities", E. H. Ashton on "Rate of change in Primate Evolution", S. B. Holt on "Dermatoglyphic Patterns", and G. A. Harrison on "Pigmentation".

Each paper has had added to it a few paragraphs indicating more recent developments in the field. □

. . . and baboons

T. H. Clutton-Brock

Social Dynamics of Gelada Baboons. (Contributions to Primatology, Volume 6.) By Robin and Patsy Dunbar. Pp. viii+157. (Karger: Basel, London and New York, 1975.) SFr.89; DM 85; \$37.25.

To casual inspection, the social system of geladas closely resembles that of hamadryas baboons: the population is divided into stable harem units usually consisting of a single mature male, around three adult females and their offspring. Younger males join unisexual troops and both harems and all-male groups form bands which share a common home range. The takeaway message of the Dunbars' monograph is that superficially similar social systems may be

the product of different behavioural arrangements. In the hamadryas, harem units are maintained by the chauvinistic activities of the male, who herds females and enforces group cohesion. In the geladas a more egalitarian situation prevails and harem units are maintained by strong social bonds between females.

The monograph provides a detailed but readable description of the anatomy of gelada social organisation. Its claim to cover group dynamics is less well justified. The study was of relatively short duration and few individuals could be recognised. As a result, virtually all interpretation of group dynamics is based on selected evidence. A more fundamental shortcoming (although one which has been applied to many primate field studies) is the lack of theoretical orientation. This leads to a number of unfortunate effects: extensive description of trivial or obvious aspects of social behaviour, persistent attention to null hypotheses which are palpably false, and inadequate discussion of points of particular interest. The book will be of major interest only to primatologists. A stronger theoretical bent could have led to a study which was of much wider significance. □

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Molecular biology of RNA phages

J. Hindley

RNA Phages. Edited by Norton D. Zinder. Pp. viii+428. (Cold Spring Harbor Laboratory: Cold Spring Harbor, 1975.) n.p.

RNA Phages is the latest of the Cold Spring Harbor Monograph series and provides 400 pages of endeavour devoted largely to our understanding of the structure, replication, and translation of the phage genome. The RNA phages are among the smallest known viruses and since the techniques for RNA sequence analysis preceded those for DNA, they formed the logical choice for the new art of the sequencer. This emphasis is apparent in many of the contributions and adds a comfortable sense of reality to the descriptions of the various interactions which regulate the replication and translation of the viral RNA.

The fourteen chapters of this book provide a reasonably well balanced selection of current work and interest in this field. Invariably there are some omissions, and some are serious, for example the work of Weissmann *et al.* on the *in vitro* synchronised synthesis of both plus and minus RNA strands; and the extension of such methods in site-directed mutagenesis gets negligible attention. Similarly the important research of Porter *et al.* on the rebinding of Q β initiator fragments to *Escherichia coli* ribosomes fails to get any mention whatever in spite of their particular relevance to the problem of ribosome recognition of initiator regions in phage RNA.

The first chapter provides an authoritative account of the physical properties of RNA phages and their RNAs, and the second contribution reviews the genetic studies which established the presence of three cistrons (complementation groups) in RNA phages.

One of the most informative features contributing to the study of RNA phages is the ability to compare primary RNA sequences with the amino acid sequences of the phage-specified proteins. The third chapter reviews our knowledge in this direction and clearly brings out the value of such work in pin-pointing the initiation triplets, the apparent non-random use of synonymous codons, the location and effects of amber mutations and the

explanation of the 'read through' phenomenon found in phage Q β .

The fourth chapter surveys the physiology of phage attachments and penetration, and makes the point that much remains to be done in understanding these phenomena. Nearly 100 pages in the succeeding four articles are devoted to the replication of phage RNA. This makes fascinating reading and the main impression one is left with is the exquisite adaptation of the replicase complex, which contains both virus- and host-specified proteins, to the selective and controlled replication of the viral RNA. No less interesting is the elaborate control exercised on the translation of the viral RNA and this topic is discussed in the next three chapters. Again it is the level of organisation and sophistication achieved in these, the simplest of phages, which excites the wonder of the reader. The *tour de force* of the book comes in chapter thirteen in which Fiers presents the entire RNA sequence of the A-protein gene of phage MS2 and shows the value of such detail in grappling with the problems of gene organisation.

To complete the picture the morphogenesis of RNA phages, and the fact that *E. coli* is not unique in acting as host to RNA phages, are discussed in two informative contributions.

The clear message of this book is that the molecular biology of RNA phages have much wider implications into the general problems of replication and translational control, and it can be recommended to the student and specialist alike. □

Paracelsian chemistry

M. P. Crosland

The Chemists and The Word: The Didactic Origins of Chemistry. By Owen Hannaway. Pp. xiii+165. (Johns Hopkins University: Baltimore, Maryland and London, November 1975.) £6.50.

WHEN did chemistry emerge as a distinct discipline? Hermes, Tresmegistos, Moses, Aristotle, Jabir, Paracelsus, Boyle, Lavoisier and Liebig have all been candidates with their respective supporters for the title of founder of chemistry. Even if we refuse to take the earlier names in this list seriously we cannot ignore a group of contemporary historians of science who have made great claims for Paracelsus and (more convincingly) for some of his followers. They have argued that the chemical philosophy of the Paracel-

sians in the sixteenth and seventeenth centuries was not only fundamental to the development of chemistry but to science generally. It sought to comprehend all natural phenomena and thus provided an alternative to both the traditional philosophy of Aristotle and the new mechanical philosophy of Descartes. Yet the obscurantism and secrecy of some Paracelsians have hardly helped to support this claim. Their apparently arbitrary use of language made any dialogue with outsiders extremely difficult. Dr Hannaway appreciates the relevance of the Paracelsians but admits that there was an essential difference between their aims and methods and that of a rational science. He therefore attempts a synthesis and tries to pin-point the beginning of the science of chemistry in a reaction to Paracelsianism. His hero is Andreas Libavius who first appears as the enemy of Paracelsian chemistry, seen as a "counter-culture". But it was the same Libavius who became the propagandist for a new chemistry, presented in a systematic and logical form.

A full discussion of the various strands of Paracelsianism would require a book of massive proportions and we can accept the author's decision to focus on one writer, Oswald Croll, as a representative of the Paracelsian school. This choice enables him to expose some of the deficiencies of the Paracelsians and to contrast their approach with that of Libavius. Hannaway suggests that chemistry emerged not so much from Paracelsian alchemy as from a more positivistic attitude of those who tried to present a new and rational science in textbook form. This is a plausible argument but the author goes further in claiming that the writing of one particular textbook, the *Alchemia* (1597) of Libavius constituted the "invention" of chemistry. It is easier to interpret the role of textbooks in science as providing a consolidation of a new approach rather than the main instrument of a "scientific revolution", and a detailed discussion of Ramism is included to help support this argument.

I would like to be able to recommend the book to any working chemist who felt that the didactic origins of his subject were worthy of some consideration. It would be fairer, however, to recommend the book rather as a contribution to the intellectual history of the sixteenth century. With its detailed textual analysis it is more a book for the specialist. Readers may not be convinced that the work of Libavius alone marked the beginning of 'real' chemistry but the discussion provides a persuasive reminder of the metaphysical and religious context of the emergence of early modern science. □

Reference work on rabies

G. S. Turner

The Natural History of Rabies. Edited by George M. Baer. Volume 1: Pp. xvi+454. \$43.00; £21.50. Volume 2: Pp. xvi+387. \$36.00; £18.00. (Academic: New York and London, September–October 1975.)

THESE two volumes provide a comprehensive central source of information on many facets of this ancient disease. They examine the molecular biology of the virus, the pathogenesis and pathology of rabies, its diagnosis, epidemiology and its prevention in man. The contributors of the 42 chapters have in many cases either been members of, or advisors to, the World Health Organisation (WHO) expert committee on rabies. Readers active in rabies research will recognise many familiar footprints in well trodden ground.

Whether the contents always justify the title of 'natural history' is arguable since this implies a continuing record of natural phenomena. Advances in rabies research seem to be discontinuous—much of the mythology was dispelled by Pasteur but more than 60 years elapsed before the successful application of cell culture methods enabled the virus to be seen for the first time, and also permitted the first reasonably accurate estimate of its size, the physicochemical and biological properties of which are described in part I. Similar methods, described in chapter 9, enabled mechanisms of infection and growth at the cellular level to be examined.

The section on the pathogenesis of rabies recalls arguments concerning the respective roles of neural and haematogenous spread of virus to the central nervous system (CNS). Evidence is also discussed for extraneural replication of virus before and after invasion of the CNS. Latent and abortive rabies is reviewed here as well as the effects of interferon on infection. Contributors remind us, however, that the scanty histological changes observed in infected animal brain, contrast strikingly with the severe clinical signs observed in rabies, and that its pathogenesis remains "a complicated and little understood series of events".

The section on laboratory diagnosis details techniques which are already described elsewhere (see WHO monograph series No. 23, *Laboratory Techniques in Rabies*). Surprisingly the passive haemagglutination test for rabies antibody does not appear here.

Almost half the second volume is devoted to epidemiology. Individual chapters provide abundant information on rabies in each of many wild and domestic animals in both natural and experimental conditions. Wildlife data concerns several species indigenous only to the new world. No doubt the omission of global epidemiology reflects difficulties in acquiring information from underprivileged countries in which rabies is endemic.

The chapters devoted to the control of rabies in dogs, cats and other domestic animals seem to prefer vaccination to other means. Although vaccination is undeniably effective it is not a control method likely to be favoured in England at the moment. Included in the methods reviewed for the control of wildlife rabies are population reduction, vaccination, the use of gametocides and more recently the use of anticoagulants for destroying vampire bats. The methods mostly suggest directions for future research rather than immediate solution of this intransigent problem.

The final chapters deal with rabies in man, its prevention, first aid treatment, diagnosis and clinical manifestations. Recent optimistic hopes of cure of the overt disease by aggressive intensive care are also mentioned. Here too, details are given of the passive immunisation of man with anti-rabies serum as well as a highly critical review of human, postexposure vaccine therapy and the difficulties of assessing its efficacy. An appendix gives a guide to specific treatment.

Not every contribution is a model of stylistic or verbal excellence and the occasional mistake has escaped the proof reader. This is probably inevitable in a work of this magnitude and in no way detracts from its value as the best and most practical reference text on the subject that has appeared in recent times. □

Endocrine system in perspective

K. Fotherby

Comparative Vertebrate Endocrinology. By P. J. Bentley. Pp. xi+415. (Cambridge University: Cambridge, London and New York, January 1976.) Cloth £12; Paperback £4.95.

ENDOCRINOLOGY is a fascinating subject and to those of us who deal mainly with human endocrinology this fascination is increased by taking a broader view and looking at the way in which the endocrine system has evolved. For this purpose this textbook is ideal. It presents the subject matter in a read-

able way emphasising the differences between species from a functional aspect. It not only contains a wealth of information concerning the endocrinology of the various species but also contains more than enough basic information concerning the biosynthesis, metabolism and mode of action of the hormones for the book to be read and understood without the necessity of referring to a textbook of basic endocrinology.

In addition to chapters on the comparative morphology of endocrine tissues and the chemical structure and polymorphism and evolution of hormones, there are chapters dealing with the relationship between endocrinology and nutrition, calcium metabolism, the integument, osmoregulation, and reproduction. The text is illustrated with many excellent diagrams.

Although there are species differences in the morphology of the endocrine tissues, and in the chemical structure and biological and immunological behaviour of the hormones, many of these variations are such that they can often be placed into categories corresponding to the major systematic groups of vertebrates. These species differences suggest an orderly evolutionary change. The pathways of hormone synthesis, mechanisms of secretion and mode of action seem to be similar in all vertebrates although the nature of the stimulus responsible for the secretion of a particular hormone may vary according to the physiological role in which the hormone may be involved.

The longest chapter in the book is that devoted to reproduction. This is not surprising since the reproductive process shows a wide diversity of morphological and physiological characteristics, and the latter requires the co-ordination of many different events which are controlled by a number of different hormones. In spite of the wide variation in the morphological and physiological aspects of reproduction, the nature of the hormones involved varies little. The gonadal steroid hormones have the same structure in all vertebrates. More variation is shown by the gonadotrophins; and it is of interest that in most tetrapods the pituitary secretes two gonadotrophins, luteinising hormone and follicle-stimulating hormone, whereas in some vertebrates e.g. fishes, only a single gonadotrophin is secreted.

Although written as a textbook for graduate and undergraduate courses in comparative endocrinology, it should be read by all interested in endocrinology. It will be useful as a reference book for research workers, and the availability of a paperback edition may ensure a wide sale for this excellent book. □

All you need to know about H-2

Hilliard Festenstein

Biology of the Mouse Histocompatibility-2 Complex: Principles of Immunogenetics Applied to a single system. By Jan Klein. Pp. xii+620. (Springer: Berlin and New York, 1975.) DM97.60; \$42.

DURING the past decade, the science of immunogenetics as applied to the major histocompatibility system, previously regarded as a somewhat esoteric area of scientific endeavour, has come into its own. Its rate of development has been unprecedented and it has provided new insight into such diverse biological problems as transplantation and cancer immunology at one end of the spectrum to those which are not even immunological at all—like hormonal control and the genetics of infertility—at the other end of the spectrum. It represents the key to the understanding of antigen recognition, cell interaction, embryonic development and differentiation, and processes involved in the pathogenesis and manifestation of a variety of diseases. It has become important not only for the basic scientist but also for clinicians—rheumatologists, neurologists, nephrologists, obstetricians, endocrinologists, to mention but a few.

The major histocompatibility system is the most complex and polymorphic system geneticists have had to cope with and the more that is known about it, the more important it seems to become. Jan Klein's exhaustive treatise on the immunogenetics of H-2 is thus a very welcome and timely arrival. It starts with an introduction dealing with the history of transplantation immunogenetics and the geneology and origin of the inbred and congenic laboratory mouse strains; there follow detailed descriptions of the methods and the current status of H-2 serology. In the section dealing with histocompatibility, there is a short introduction to transplantation immunology followed by a discussion on the methods of histocompatibility testing and effects, comparing those of the H-2 system with various others—the erythrocyte (Ea) alloantigen loci, Ly, virus-associated alloantigen loci and non-H-2 histocompatibility loci.

The section on the principles of genetic analysis, recombination and mutation in the H-2 complex and the genetics of the H-2 linked loci will be

of particular value to those researchers entering the field; it contains a clear account of the genetic terminology and approach basic to an understanding of this science. There is also a comprehensive section on the biochemistry of the H-2 gene products. The book is reasonably up to date and even includes discussions on the recently discovered complement gene in H-2 and the Ia specificities, as well as the newly described X locus. It ends with a short speculative chapter on the function and evolution of the H-2 complex; and there is a chronological table of the major events in the history of the H-2 system which is interesting and puts the author's own contributions into perspective. The references are an invaluable part of this book and will provide researchers with a rapid and comprehensive list of the major publications in this fascinating and new field.

This book will probably find a place on the shelf of every laboratory seriously engaged in the study of immunogenetics. □

Indispensible guide to tropical pollution

C. M. Yonge

Tropical Marine Pollution. (Elsevier Oceanography Series, 12.) Edited by E. J. Ferguson-Wood and R. E. Johannes. Pp. ix+192. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.65; \$26.95.

WE may be grateful for the original idea of the late E. J. Ferguson-Wood resulting in the production of this book under the final editorship of R. E. Johannes, who is also responsible for writing much of the most important sections.

Although temperate seas have been the initial sufferers from pollution, the basically more susceptible tropical waters are all too rapidly becoming similarly afflicted. And what may be effective preventive measures in the former regions may not be applicable in the tropics. In so many different ways involving chemical and physical characters, community structure and biological processes, responses to pollution in the tropics differ significantly from those in temperate waters.

The second and longest chapter comprises the fullest available study of pollution and degradation of coral reef communities. Writing with a great wealth of experience, Dr Johannes covers every aspect of the problem from the effects of sedimentation to those of dredging, from those of bad land management to those of sugar mill discharge and of sewage outflow,

the latter so dramatically demonstrated at Kaneohe Bay to the north of Honolulu where I once collected from immaculate reefs. He finally discusses effects of oil and thermal pollution, and those of abnormal salinities. These last are produced by desalination plants, an analysis by R. H. Chesher of the impact of one at Key West forming the final chapter.

What is known about the impact of man on mangroves—it is estimated that some of those defoliated during the Vietnam war may take a century to recover—is succinctly reviewed by W. E. Odum and R. E. Johannes. There is a most useful account by J. C. Zieman of the effect of pollution on tropical eel grass communities, aptly described as "nurseries, food producers and stabilisers of the sea floor." The same author also collaborated with Ferguson-Wood in a description of the effects of thermal pollution by a power station most unsuitably sited in Biscayne Bay, Florida and involving the destruction of both sea grass beds and mangroves.

Although there is much of value in all chapters, those contributed by Dr Johannes are of over-riding importance greatly enhanced by his personally assembled list of references occupying 28 pages. This is a short and expensive book but is contains a wealth of information valuable to all, but surely indispensable to those confronted by the almost daily growing problems of pollution in tropical seas. □

Multidisciplinary approach to pollution

N. W. Moore

Pollutants and Animals: A Factual Perspective. By F. Moriarty. Pp. 140. (Allen and Unwin: London, September 1975.) £5.65.

THIS short book is a lucid account of the facts which have to be taken into account by those who study the effects of pollutants on living organisms. Probably more is known about the pollution effects of the organochlorine insecticides than any other chemical group, and so Dr Moriarty uses the organochlorines as the example on which to base his pertinent conclusions. About 80% of the book is about these compounds and the chemically related polychlorobiphenyls (PCBs), the remaining 20% being devoted to the effects of heavy metals, and a brief general review of pollution in air, water and on land. Radioisotopes are covered in two pages. At first sight the subject matter of the book seems unbalanced, but the advantages of using the organo-

chlorines as an example outweigh this disadvantage.

Pollutants and Animals is accurate, clearly written and very readable. Technical terms such as axon, isomer and isotope are defined and so it can be read by the informed layman as well as by scientists with a professional interest in pollution research and control, or in teaching or learning about pollution. Dr Moriarty shows how ideas have progressed in recent years, and, by pointing out significant gaps in knowledge, also succeeds in putting over the authentic 'feel' of doing research in this field. Studies which should be extended include those on the possible biological effects of pesticide metabolites, the behaviour of DDT in mud, the extent to which DDT is converted into DDE and transformed to PCBs in the physical environment, and laboratory studies on the interactions of different pollutants on fish.

Dr Moriarty's comments on the limitations of the LD₅₀ test are salutary for those who rely too much on this particular indicator of hazard. The desirability of obtaining more residue data when toxicological tests are made, is another practical requirement to

which Dr Moriarty draws attention.

Conventional wisdom has it that organochlorine insecticides always become concentrated in food chains. In his critique of the pioneer work of Hunt and Bischoff at Clear Lake, Dr Moriarty shows how that hypothesis has had to be modified in the light of subsequent research. Similarly, it is commonly believed that populations of birds of prey have declined as the result of sublethal effects of organochlorines. Dr Moriarty, in a perceptive review of this very complicated subject, shows how lethal and sublethal effects have varied in their relative importance between different species.

This book should be of great interest to those concerned with the organisation of science. It demonstrates not only the desirability, but the necessity of relating chemistry, toxicology and population dynamics in order to provide the scientific base for pollution control. The multidisciplinary team of which Dr Moriarty was a distinguished member, was a casualty of the split of the Nature Conservancy. Some way must be found of supporting similar teams in the future. Anyone reading this excellent book will come to appreciate why. □

Food from the sea

G. E. Fogg

Marine Photosynthesis: With Special Emphasis on the Ecological Aspects. (Elsevier Oceanography Series, 13.) By E. Steemann Nielsen. Pp. ix+141. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.52.00; \$21.75.

PROFESSOR Steemann Nielsen has contributed more than any other person to our knowledge of photosynthesis in the sea. His introduction in 1952 of the radiocarbon technique gave us a simple and sensitive means, which has been very widely used, for measuring the rate of photosynthesis in natural waters; and his laboratory experiments have deepened our understanding of the manner in which the photosynthetic activity of phytoplankton organisms varies in response to conditions in the sea. In this book, the first to be devoted to a kind of photosynthesis which provides at least half the Earth's supply of food materials, he brings together these contributions and sets them against the background of modern ideas about the mechanism of the photosynthetic process and its evolutionary history.

This background is sketched in the first two chapters; there follow discussions of underwater daylight, the units in which irradiance should be expres-

sed, the kinds of photosynthetic organisms in the sea, the effects of various factors on their photosynthetic activity, the interrelationships of photosynthesis and respiration, and physiological adaptation to irradiance and temperature. The later chapters deal with photosynthesis *in situ*, and the primary production of the sea and its importance to man. It is a pity that in this account the author should have confined himself rather strictly to presenting his own work and avoided discussion of controversial matters such as the importance of extracellular products of photosynthesis.

Several independent groups of investigators now believe that they have demonstrated that healthy populations of natural phytoplankton frequently release as much as half of their photosynthetic product directly into the surrounding water. This much is briefly mentioned but after a longer discussion of the errors which may arise in the determination of extracellular products of photosynthesis the reader may be left with an impression, which the facts do not actually justify, that this important phenomenon is no more than an experimental artefact. Similarly, there is insufficient consideration of the implications of experiments by other workers on temperature adaptation.

Nevertheless, this is a wide-ranging and authoritative account which will be valuable and stimulating to students and research workers alike. □

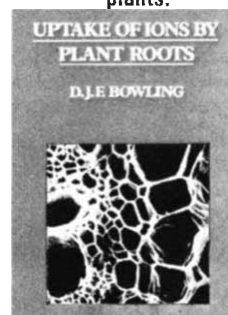
MUTATION RESEARCH

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Biological problems of the deep sea

N. B. Marshall

*Physiological Aspects of Deep-Sea
Biology* By A. G. Macdonald. Pp.
xi+450. (Cambridge University:
Cambridge and London, October 1975.)
£18.50.

MONOGRAPHS of the Physiological Society "... review and assess important and neglected areas of physiology". The physiological aspects of this monograph are centred largely on the effects of hydrostatic pressure, which is certainly an important and neglected area of physiology. After the early experiments of Regnard and Certes in the 1880s, little was done until the late 1920s and 30s, as may be seen by reference to Cattell's (1936) *Biological Review*. The more recent and considerable advances in our knowledge of the effects of pressure on organisms are evident in Symposium XXVI (1972) of the Society of Experimental Biology and in an American Symposium (1972) entitled *Barobiology and the Experimental Biology of the Deep Sea* (Univ. North Carolina). Dr Macdonald was a contributor to both symposia. Besides his concern with the effects of pressure on the activity and metabolism of oceanic animals, he has also been involved in the design of apparatus for recovering deep-sea animals without pressure change.

In his brief introduction to the physical nature and inhabitants of the deep sea, the author devotes most space to the few species of animals, such as the ostracod *Gigantocypris* and the pogonophoran worm *Siboglinum*, that have been used by physiologists. There follow chapters on the molecular, biochemical, and physiological aspects of high hydrostatic pressure. Between these chapters and one entitled *Deep-Sea Bio-engineering* there are two dealing with sensory physiology and buoyancy in the deep sea and deep-sea nutrition.

In his competent reviews of the effects of pressure from the molecular to organ system level, it is clear from more than the bibliography that most of the author's research has been on whole animals. When dealing with molecular aspects, he concludes that high pressure is of considerable importance in the molecular biology of deep-sea organisms but adds these cautionary words: "The volume changes and pressure effects which have been described were largely

derived from *in vitro* systems and we have eventually to tackle the functional, living state. It is conceivable that a great deal of pressure sensitivity may disappear when an isolated protein is returned to its physiological environment. It is equally possible that the pressure sensitivity of intact animals will be greater than their isolated parts through amplification processes at the kinetic and integrated levels of organisation." Later he remarks that "... the intact organism may possess compensatory mechanisms which are absent in an isolated organ. Experiments with intact animals will help to identify some of their pressure-sensitive components." These quotations also show that the author writes well (although he could be more of a 'which hunter').

Thirty years ago, Sir Frederick Russell foresaw that physiologists would be eager to study deep-sea organisms when proper facilities were available. That physiologists are now able to take their equipment to sea is abundantly plain in Dr Macdonald's review of sensory physiology and buoyancy. Perhaps the outstanding contribution of other sea-going physiologists towards a proper understanding of deep-sea nutrition has been to measure rates of metabolism. How, for instance, do the changing pressures and temperatures that are experienced during vertical migrations affect the rate of oxygen consumption of deep-sea animals? Recently, *in situ* respirometry of benthic communities and benthopelagic fishes has shown that the pace of deep-sea life is much slower than that in shallow waters. But deep-sea conditions can be simulated in the laboratory, and what can be achieved by high pressure systems is well reviewed by Dr Macdonald in his last chapter, which also deals with the physiology of deep-sea man and the roles of deep-sea vehicles and surface ships.

In a comprehensive book of this kind errors are bound to occur, but one that is presumably not due to the author concerns the caption of Fig. 1.21 on page 28. The photograph is not of a rat-tail (macrourid) but of a chimaerid species.

The book contains a full bibliography (up to 1975). The references for each chapter are kept separate but 'pressurised' together at the end of the book, which makes transfers from textual references to bibliographies irritating to the reader. Finally, I agree with the publishers' statement that "The book will be of great value to all interested in marine physiology and the biological problems of the deep sea" but wonder how many of "all interested" will be able to buy it. □

articles

α -Chain contacts in the polymerisation of sickle haemoglobin

Ruth E. Benesch, Suzanna Yung, Reinhold Benesch & John Mack

Department of Biochemistry, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Rose G. Schneider

Department of Pediatrics, University of Texas Medical Branch, Galveston, Texas 77550

Five new double-mutant haemoglobins composed of β^S chains and α chains with different substitutions, which are located at the surface of the tetramer, have been prepared. Although all the hybrids are more soluble than deoxyhaemoglobin S, the individual differences between these molecules make it possible to evaluate several regions on the α chains for intermolecular contacts in the polymerisation of deoxyhaemoglobin S.

THE discovery that the aggregation of deoxyhaemoglobin S (HbS) involves the formation of tightly packed helical rods^{1,2} shows that multiple contact points must cooperate in the formation of the individual helices as well as in the interaction between adjacent tubules to give tactoids³. Identification of these contact points is facilitated by investigating the behaviour of haemoglobins which, in addition to the β^S valine mutation, have others elsewhere on the surface of the molecule. Only two of these have so far been investigated—HbC Harlem^{4,5} and HbS Memphis—and in both cases the additional substitution diminishes the tendency to sickle⁴⁻⁶. This implicated β^{73} and α^{23} in contact formation between adjacent molecules. To widen this approach we have prepared five new double-mutant haemoglobins which carry the β^S valine substitution together with an additional one on the α chains. This facilitates a direct comparison of the effect of changing specific residues on the α chain on the properties of haemoglobins carrying the β^S substitution.

Preparation of hybrid haemoglobins

Haemoglobin was prepared from fresh human blood as described previously⁷. The mutant haemoglobins used in this investigation are listed in Table 1. HbS was obtained from a single homozygous donor by exchange transfusion. It was isolated by chromatography on CM-Sephadex (C-50) using a salt gradient of 0→0.1 M NaCl in 0.05 M phosphate buffer, pH 6.7. All the other mutant haemoglobins were separated from the haemolysates on DEAE-Sephadex (A-50) with a pH gradient of Tris buffer¹³. After column chromatography the haemoglobin solutions were concentrated using Amicon or collodion membranes. The purity of the isolated mutant haemoglobins was checked by acrylamide gel electrophoresis¹⁴.

Hybrid molecules containing two β^S chains and two α chains derived from the mutant haemoglobin were prepared by incubating the α -chain mutant haemoglobin with a twofold excess of HbS in 0.2 M acetate buffer, pH 4.8, for 4 h at 0 °C as

described previously¹⁵. The hybrids were again isolated by chromatography on DEAE or CM-Sephadex. HbS alone was subjected to identical procedures to serve as a control.

The identity and purity of the hybrids was established by electrophoresis both of the intact hybrid haemoglobins¹⁶ and of the individual polypeptide chains¹⁷. Desarg HbS was prepared as described for Desarg HbA¹⁸. One mol of (arginine+ornithine) was liberated per $\alpha\beta$ dimer and the protein was homogeneous on acrylamide gel electrophoresis. The oxygen equilibrium curves of all haemoglobins, including the hybrids, were measured at 20 °C and pH 7.30 as described previously^{19,20}. Solubility was determined as a function of ionic strength in phosphate buffer, pH 6.8, by a modification of Itano's method^{21,22} both in the presence and absence of dithionite. This method was chosen, rather than gelation measurements because it requires much

Fig. 1 Electrophoretic patterns of native and hybrid haemoglobins and their constituent globin chains on cellulose acetate. Tris-EDTA borate (TEB) buffer, pH 8.5, for haemoglobins; TEB buffer, pH 8.9, containing 2-mercaptoethanol and 6 M urea, for globins.

Hb & Structure	Cellulose Acetate pH 8.5				Globin Chains pH 8.9			
	+	A	S	C	+	β^A	β^S	α^A
AS								
Sealy $\alpha 47$ Asp → His								
Hybrid $\alpha 2$ Sealy $\beta 2S$								
Shimonoseki $\alpha 54$ Gln → Arg								
Hybrid $\alpha 2$ Shimo $\beta 2S$								
Montgomery $\alpha 48$ Leu → Arg								
Hybrid $\alpha 2$ Mont $\beta 2S$								
G-Philadelphia $\alpha 68$ Asn → Lys								
Hybrid $\alpha 2$ G-Phila $\beta 2S$								
I $\alpha 16$ Lys → Glu								
Hybrid $\alpha 21$ $\beta 2S$								
AC								

Table 1 Properties of mutant haemoglobins

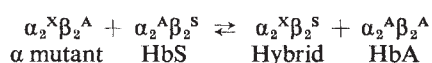
Mutant haemoglobin	Structure	Ref.	% Total haemoglobin
Hb Sealy (hasharon)	$(\alpha^{47} \text{ Asp} \rightarrow \text{His})_2 \beta_2^A$	8	20
Hb Shimonoseki	$(\alpha^{54} \text{ Glu} \rightarrow \text{Arg})_2 \beta_2^A$	9	24
Hb Montgomery	$(\alpha^{48} \text{ Leu} \rightarrow \text{Arg})_2 \beta_2^A$	10	23
HbI	$(\alpha^{16} \text{ Lys} \rightarrow \text{Glu})_2 \beta_2^A$	11, 12	14
HbG Phila	$(\alpha^{68} \text{ Asn} \rightarrow \text{Lys})_2 \beta_2^A$	9	100*
HbSG Phila	$(\alpha^{68} \text{ Asn} \rightarrow \text{Lys})_2 (\beta^6 \text{ Glu} \rightarrow \text{Val})_2$	9	21

*The blood of this patient contained only HbG Phila and Hb H.
The latter was precipitated quantitatively leaving HbG Phila in solution.

smaller amounts of material. Studies of chemically modified HbS have shown that these two methods give parallel results²³.

Characterisation of double-mutant molecules

Hybridisation between HbS and the α -mutant haemoglobins can be represented by the following equation:



The electrophoretic mobilities of all the native and hybrid haemoglobins are shown in diagrammatic form in Fig 1. Four of the mutant haemoglobins, that is, haemoglobins Sealy, Shimonoseki, Montgomery and G Phila, have the same charge as HbS. Therefore, only three components are isolated after hybridisation, that is, the hybrid and HbA in equal amounts and a fraction of intermediate mobility containing both HbS

($n=2.4-2.6$) and with a normal oxygen affinity. (2) HbS reisolated after hybridisation with HbA has the same solubility as the untreated protein. (3) In the case of one of the hybrids, that is $\alpha_2^{\text{GPhil}} \beta_2^S$, it was possible to compare the tetramer prepared *in vitro* by hybridisation of HbG Phila and HbS with a naturally occurring one isolated from the blood of a patient who is heterozygous for HbG Phila and homozygous for HbS⁸. Since the natural and the synthetic hybrid gave the same result (Table 2) it can be assumed that the procedure for preparing the other hybrids has no effect on their solubility *per se*.

Solubility of mutant sickle haemoglobins

The effect of different substitutions in the α chain on the solubility of deoxyhaemoglobin S is shown in Fig. 2. Clearly all the double-mutant haemoglobins are significantly more soluble in strong salt than deoxyhaemoglobin S. This is in sharp contrast to the parent α -mutant haemoglobins which all have the same solubility as deoxyhaemoglobin A, that is their solubility is 10^{-5} M at an ionic strength of 5.70.

Since the most characteristic property of HbS is the large difference in the solubility of the oxy and deoxy forms, this value is used in Table 2 to compare the solubilising effect of the various α -chain mutations. Their influence on the solubility of the hybrid molecules must be due to interference with the polymerisation characteristic of deoxyhaemoglobin S because no solubilising effect of the α -chain substitution is found in the parent haemoglobin.

It is striking that a chemical modification of the α chains which favours the oxy conformation of haemoglobin¹⁸, that is, removal of the C-terminal arginine residues, has little effect on the solubility of deoxyhaemoglobin S (Fig. 2).

The inhibitory effects of the different α -chain substitutions on the aggregation of HbS are not simply related to the alteration in charge. Thus, the Sealy and G Phila hybrids have the same charge and a very different solubility while the HbI and G Phila hybrids are almost equally soluble, but differ by six charges per tetramer.

It therefore seems that the synthetic hybrids represent a new approach to locating contact points provided by the α chains in the polymerisation of deoxy HbS. Such information is essential to restrict the number of possible orientations of the tetramers within the helix²⁴⁻²⁷.

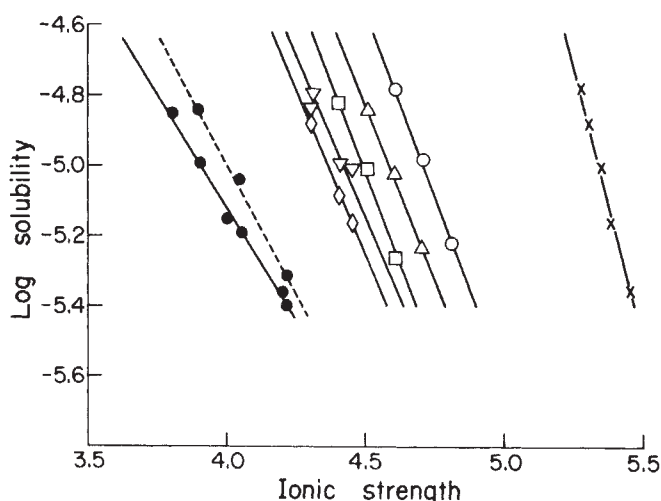


Fig. 2 Solubility of HbS and double-mutant haemoglobins. All measurements in potassium phosphate buffer, pH 6.8, at 25 °C. The solubility is given in mol of Hb tetramer per l of solution in the presence of solid phase. ●—●, S deoxy; ●—●—●, desarg S deoxy; ×, S oxy; ○, $\alpha_2^{\text{Sealy}} \beta_2^S$ deoxy; △, $\alpha_2^{\text{Shimo}} \beta_2^S$ deoxy; □, $\alpha_2^{\text{Mont}} \beta_2^S$ deoxy; ▽, $\alpha_2^{\text{GPhila}} \beta_2^S$ deoxy; ◇, $\alpha_2^{\text{I}} \beta_2^S$.

and unchanged α mutant. In the case of HbI, on the other hand, all four components of the hybridisation mixture can be eluted separately. The hybrid haemoglobins comprised from 15 to 20% of the total. The electrophoretic patterns of the constituent globin chains of both the native haemoglobins and the hybrids are also shown in Fig. 1. The α chains of the four haemoglobins that resemble HbS electrophoretically all move cathodically to α^A , but the α chain of HbI moves anodically to α^A . The mobility of the α^I globin happens to be the same as that of β^S , so that α^I and β^S form only one band. None of the hybrid haemoglobins showed any appreciable contamination with α^A or β^A globin chains.

The structural and functional integrity of the hybrid tetramers was tested in several ways. (1) They bind oxygen cooperatively

Fig. 3 Schematic model of the haemoglobin molecule showing the locations of the amino acid substitutions.

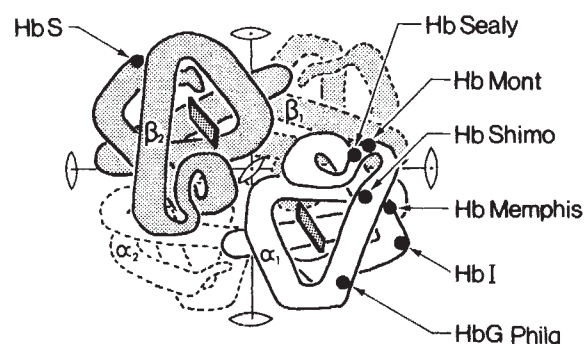


Table 2 Effect of deoxygenation on solubility

Haemoglobin	Position of α substitution	Ionic strength at which the solubility is 10^{-5} M		
		Oxy	Deoxy	(Oxy-deoxy)
$\alpha_2^A\beta_2^S$		5.33	3.90	1.43
$\alpha_2^{\text{Sealy}}\beta_2^S$	47	5.28	4.70	0.58
$\alpha_2^{\text{Shimonoseki}}\beta_2^S$	54	5.28	4.58	0.78
$\alpha_2^{\text{Montgomery}}\beta_2^S$	48	5.43	4.48	0.95
$\alpha_2^{\text{G Phila}}\beta_2^S$ (synthetic)	68	5.50	4.42	1.08
$\alpha_2^{\text{G Phila}}\beta_2^S$ (natural)	68	5.50	4.42	1.08
$\alpha_2^I\beta_2^S$	16	5.50	4.35	1.15

The positions of the α substitutions are shown in Fig. 3. It should be noted that all of them are on or near the surface of the molecule. It is clear, however, that there is considerable variation between the effect of the different substitutions on polymerisation. The largest increase in solubility is observed with $\alpha_2^{\text{Sealy}}\beta_2^S$ with a substitution in the CD bend. Therefore, an intermolecular bond is probably located at this position. Moreover, the magnitude of the effect makes it likely that this bond is responsible for a large portion of the contact energy in this region. Conversely, the small increase in solubility due to the replacement of α^A by α^I chains suggests that here the substitution breaks only one of many bonds which participate in this contact region. This is in agreement with the conclusions reached by Levinthal *et al.*²⁸ Figure 2 shows that the hybrids with α chains from haemoglobins Sealy and Montgomery have significantly different solubilities, although the substitutions are on adjacent loci ($\alpha 47$ and $\alpha 48$). Inspection of the three-dimensional model of haemoglobin reveals, however, that although residue 47 is on the surface of the molecule pointing outwards, residue 48 is oriented in the opposite direction.

The α mutants available to us have only permitted the preparation of hybrids with substitutions as far as the E helix (Fig. 3). We hope that gifts of α mutants with substitutions elsewhere on the α chain will make it possible to explore contact points in the remainder of this chain.

This work was supported by grants from the US Public Health Service, the US National Science Foundation and the National Foundation-March of Dimes. R. B. is a recipient of a research career award from the National Heart and Lung Institute. For blood samples we thank Drs Jack B. Alperin, Thomas Hosty, Mary Jane Grey, James Wolff, and Ronald Rieder.

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- 1 Magdoff-Fairchild, B., Swerdlow, P. H., and Bertles, J., *Nature*, **239**, 217-219 (1972).
- 2 Finch, J. T., Perutz, M. F., Bertles, J. F., and Dobler, J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 718-722 (1973).
- 3 Huntsman, R. G., and Lehmann, H., *Br. J. Haemat.*, **28**, 437-444, (1974).
- 4 Bookchin, R. M., Nagel, R. L., and Ranney, H. M., *J. biol. Chem.*, **242**, 248-255 (1967).
- 5 Bookchin, R. M., and Nagel, R. L., *J. molec. Biol.*, **60**, 263-270 (1971).
- 6 Kraus, L. M., Miyaji, T., Iuchi, I., and Kraus, A. P., *Biochemistry*, **5**, 3701-3708 (1966).
- 7 Benesch, R. E., Benesch, R., Renthall, R. D., and Maeda, N., *Biochemistry*, **11**, 3576-3582 (1972).
- 8 Schneider, R. G., Ueda, S., Alperin, J. B., Brimhall, B., and Jones, R. T., *Am. J. Human Genet.*, **20**, 151-156 (1968).
- 9 Schneider, R. G., *et al.*, *Symp. Abnormal Hemoglobins Thalassemia*, Istanbul, 1974 (in the press).
- 10 Brimhall, B., *et al.*, *Biochim. biophys. Acta*, **379**, 28-32 (1975).
- 11 Ranney, H. M., O'Brien, C., and Jacobs, A. S., *Nature*, **194**, 743-745 (1962).
- 12 O'Brien, C., Gray, M. J., and Jacobs, A. S., *Am. J. Obstet. Gynec.*, **88**, 816-822 (1964).
- 13 Huisman, T. H. J., and Dozy, A. M., *J. Chromat.*, **19**, 160-169 (1965).
- 14 Davis, B. J., *Ann. N. Y. Acad. Sci.*, **121**, 404-427 (1964).
- 15 Ranney, H. M., Benesch, R. E., Benesch, R., and Jacobs, A. S., *Biochim. biophys. Acta*, **74**, 544-547 (1963).
- 16 Schneider, R. G., in *The Detection of Hemoglobinopathies*, 11-14 (edit. by Schmidt, R. M., Huisman, T. H. J., and Lehmann, H.), (Chemical Rubber Co., Cleveland, 1974).
- 17 Schneider, R. G., *Clin. Chem.*, **20**, 1111-1115 (1974).
- 18 Antonini, E., Wyman, J., Zito, R., Rossi-Fanelli, A., and Caputo, A., *J. biol. Chem.*, **236**, PC60-63 (1961).
- 19 Benesch, R., Macduff, G., and Benesch, R. E., *Analyt. Biochem.*, **11**, 81-87 (1965).
- 20 Benesch, R. E., Benesch, R., and Yung, S., *Analyt. Biochem.*, **55**, 245-248 (1973).
- 21 Itano, H. A., *Archs Biochem. Biophys.*, **47**, 148-159 (1953).
- 22 Benesch, R., Benesch, R. E., and Yung, S., *Biochem. biophys. Res. Commun.*, **55**, 261-265 (1973).
- 23 Benesch, R., Benesch, R. E. and Yung, S., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1504-1505 (1974).
- 24 Wishner, B. C., and Love, W. E., *Proc. first natn. Symp. Sickle Cell Disease*, DHEW Publ. No. 75-723, 85-87 (US Government Printing office, Washington, DC, 1974).
- 25 Levinthal, C., Kahn, P., Wodak, S. J., and Dadivanian, A. K., *Proc. first natn. Symp. Sickle Cell Disease*, DHEW Publ. No. 75-723, 89-91 (US Government Printing office, Washington DC, 1974).
- 26 Edelstein, S. J., Dykes, K. W., Telford, J. N., and Crepeau, R. H., *Proc. first natn. Symp. Sickle Cell Disease*, DHEW Publ. No. 75-723, 129-130 (US Government Printing Office, Washington DC, 1974).
- 27 Magdoff-Fairchild, B., Greene, P. E., and Bertles, J. F., *Proc. first natn. Symp. Sickle Cell Disease*, DHEW Publ. No. 75-723, 131-132 (US Government Printing Office, Washington DC, 1974).
- 28 Levinthal, C., Wodak, S. J., Kahn, P., and Dadivanian, A. K., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1330-1334 (1975).

Bacterial cloning of plasmids carrying copies of rabbit globin messenger RNA

T. H. Rabbitts

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

DNA copies of rabbit globin mRNA have been inserted into the plasmid mini-ColEI. After addition of poly(dT) tails, the plasmid was used to prime the copying of mRNA by reverse transcriptase. Mini-ColEI carrying globin sequences were denatured and annealed with mini-ColEI tailed with poly(dA). Functional plasmids containing the synthesised globin sequences were isolated by transfection in E. coli.

It has been demonstrated that eukaryotic DNA can be cloned in bacteria after insertion into plasmids¹⁻⁴ or bacteriophage genomes⁵. These experiments indicate that it should be possible

to clone any eukaryotic gene in bacteria and identify those bacteria carrying the gene by screening procedures involving hybridisation with radioactive messenger RNA (mRNA). This cloned DNA will facilitate the study of the sequence organisation and structure of eukaryotic genes. At present, bacterial cloning of defined eukaryotic protein-specifying genes has only been achieved with the multiple-copy histone genes⁶. The greatest proportion of genes, however, are found as single copies in the eukaryotic genome (for review see ref. 7).

Unless substantial purification of such genes can be achieved *in vitro*, cloning of individual plasmid-gene chimaeras (made from unfractionated eukaryotic DNA) will be difficult since specific clones are likely to be present in the population at the

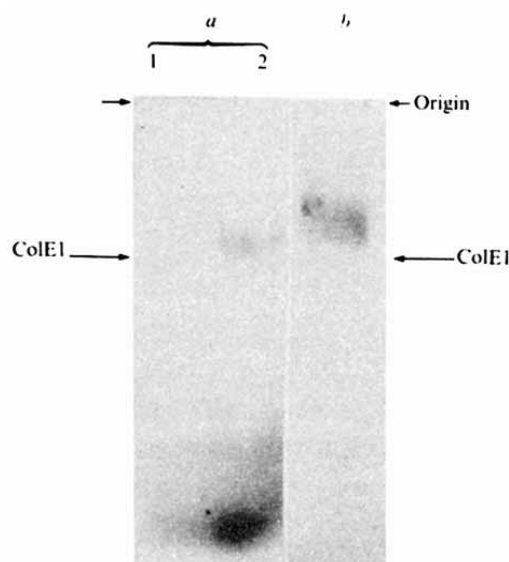


Fig. 1 Analytical Agarose gel electrophoresis of terminal transferase and reverse transcriptase activity with mColE1 DNA. *a*, 0.5 μ g intact mColE1 DNA (panel 1) or 0.5 μ g *Eco*RI-digested mColE1 DNA (panel 2) were incubated with 1 μ Ci 32 P-dTTP (New England Nuclear, 100 Ci mmol $^{-1}$) and 2.5 μ g terminal transferase (P.L. Biochemicals, Lot No. 34421, specific activity 5,260 U mg $^{-1}$) in 10 μ l reaction volume containing 100 mM potassium cacodylate (pH 7), 7.5 mM potassium phosphate (pH 7), 8 mM MgCl $_2$, 2 mM 2-mercaptoethanol. After 1 h at 37 $^{\circ}$ C, the reaction was terminated by addition of EDTA to give 10 mM and sucrose was added to 20% (w/v): the mixture was loaded on a 1% Agarose (w/v) vertical slab gel. Electrophoresis was for 1 h at a constant current of 40 mA. (The gel buffer was 20 mM sodium acetate, 40 mM Tris-acetate (pH 8.3), 0.2 mM EDTA.) An autoradiograph of the gel was made using Kodak Auto-Process X-ray film. Unreacted 32 P-dTTP appears (near the anode) at the bottom of the autoradiograph. *b*, 2 μ g mColE1 DNA tailed with poly(dT) (prepared as in legend to Fig. 2) plus 1 μ g rabbit globin mRNA were incubated in 50 μ l containing 50 mM Tris-HCl (pH 8.3), 20 mM dithiothreitol, 6 mM MgCl $_2$, 60 mM NaCl, 500 μ M dATP, dCTP, dGTP, dTTP, 1 μ Ci 32 P-dCTP, 2.5 μ g actinomycin D and 0.16 μ g reverse transcriptase (specific activity 29,316 U mg $^{-1}$). After 2 h at 37 $^{\circ}$ C the reaction mixture was diluted with an equal volume of 0.6 M NaCl, 0.02 M sodium acetate (pH 5) and passed over a small column (in a 1-ml plastic pipette) of Sephadex SP-50 to remove the unreacted nucleotides. The breakthrough peak was precipitated with ethanol (-20° C, 18 h). The precipitate was dissolved in gel buffer, electrophoresed and the gel autoradiographed as in Fig. 1*a*. The anode is at the bottom of the gel.

level of 1 cell in 10^6 . Methods for isolating clones such as the modified sib selection procedure used to identify bacteria carrying plasmids with histone genes⁶ is unattractive for low frequency genes since the number of screening selections would need to be very large. A report by Rougeon *et al.*⁸ has demonstrated the insertion of double-stranded DNA copies of rabbit globin mRNA into the plasmid pCR1. Using different methods from those described by Rougeon *et al.*⁸, we have inserted double-stranded complementary DNA copies of rabbit globin mRNA into the plasmid mini-ColE1 (mColE1)⁹. The method involves a minimum of manipulations and should be suitable for producing such plasmids from any mRNA containing poly(A). The procedure is a modification of the A-T tail method^{10,11} and utilises poly(dT) tails, added to mColE1, as primer for reverse transcriptase copying of globin mRNA into DNA. Double-stranded globin DNA is produced by repair *in vivo* of the single-stranded complementary DNA (cDNA) in the plasmid.

Preparation of plasmids containing globin DNA

The plasmid mColE1 possesses a single site for cleavage by the restriction endonuclease *Eco*RI (ref. 9). Digestion of the plasmid with this enzyme therefore yields a uniform population of linear

molecules with an identical point of breakage. It has previously been shown that the enzyme terminal transferase adds nucleotides to 3'-termini of double-stranded DNA molecules¹⁰⁻¹² but that the efficiency of the process is greatest in the presence of a free 3'-OH group. Nevertheless, the ability of terminal transferase to add dT-residues to the unmodified ends of *Eco*RI-digested mColE1 was tested. A sample of mColE1 digested with *Eco*RI was incubated with terminal transferase plus 32 P-dTTP. The products were analysed by Agarose gel electrophoresis (Fig. 1*a*). The enzyme adds dT residues to the DNA to produce a heterogeneous size range of elongated molecules (Fig. 1*a*, panel 2). Intact circular plasmid DNA shows no change when incubated with terminal transferase (Fig. 1*a*, panel 1). The size range of the poly(dT) tails was rather difficult to control, however, and this heterogeneity is presumably due to the poor and unsynchronised initiation of elongation.

The ability of the mColE1 molecules carrying poly(dT) tails to act as primer for reverse transcriptase copying of globin mRNA was tested. This transcription would be analogous to the conventional preparation procedure of cDNA from mRNA (for example see ref. 13). Fig. 1*b* shows that a further elongation of the mColE1 resulted from the reverse transcriptase activity. Thus DNA copies of globin mRNA had been added (covalently attached) to the mColE1 by reverse transcriptase.

These observations showed that a population of plasmid molecules terminated by segments of globin DNA could easily be prepared and led to the development of a procedure for the cloning of plasmids carrying these globin sequences. The general protocol for this preparation is given in Fig. 2. The procedure consisted of five steps. (1) Circular mColE1 was digested to completion with *Eco*RI; (2) the digested plasmid was tailed with either poly(dT) or poly(dA); (3) the plasmid tailed with poly(dT) was used to prime the synthesis of cDNA using globin mRNA as a template; (4) the plasmid molecules containing cDNA were further tailed with poly(dT); (5) the resultant population from step 4 was mixed with plasmid molecules tailed with poly(dA) and the mixture denatured and annealed. Transfection was carried out with *Escherichia coli*. Colicin-resistant clones were plated on nutrient agar and grown overnight at 37 $^{\circ}$ C. *E. coli* clones containing globin plasmids were finally screened by hybridisation.

The annealing procedure was carried out in two parts. First, plasmid DNA from step 4 (that is, carrying globin DNA and a further poly(dT) tail) was mixed with an excess of plasmid DNA tailed with poly(dA). This mixture was denatured and annealed at high temperature and high DNA concentration to allow specific hybridisation of the plasmid DNA strands. Second, the DNA mixture was diluted to about 5 μ g ml $^{-1}$ before incubation at 37 $^{\circ}$ C to facilitate hybridisation of the poly(dA) tail of one strand with the poly(dT) tail present at the end of the globin DNA segment on the opposite strand. Such dilution of the DNA is known to facilitate intramolecular hybridisation of poly(dA) and poly(dT) (that is circularisation) rather than intermolecular hybridisation¹⁴. Although plasmid circularisation was not demonstrated directly, it was found that production of transfectant *E. coli* was absolutely dependent on the final 37 $^{\circ}$ C annealing step. Since the reverse transcription was carried out in the presence of actinomycin D, only single-stranded globin DNA would be synthesised. It is possible that the transfection efficiency could have been improved by repair *in vitro* of the single-stranded region and subsequent *in vitro* ligation. Since globin-plasmids could be produced without such *in vitro* steps, however, repair and ligation of the plasmids was allowed to occur *in vivo*.

Identification and characterisation of bacteria carrying globin plasmids

The first stage in the identification of individual clones containing globin plasmids was growth of the transfected cells in the presence of colicin. Only cells carrying mColE1 plasmids

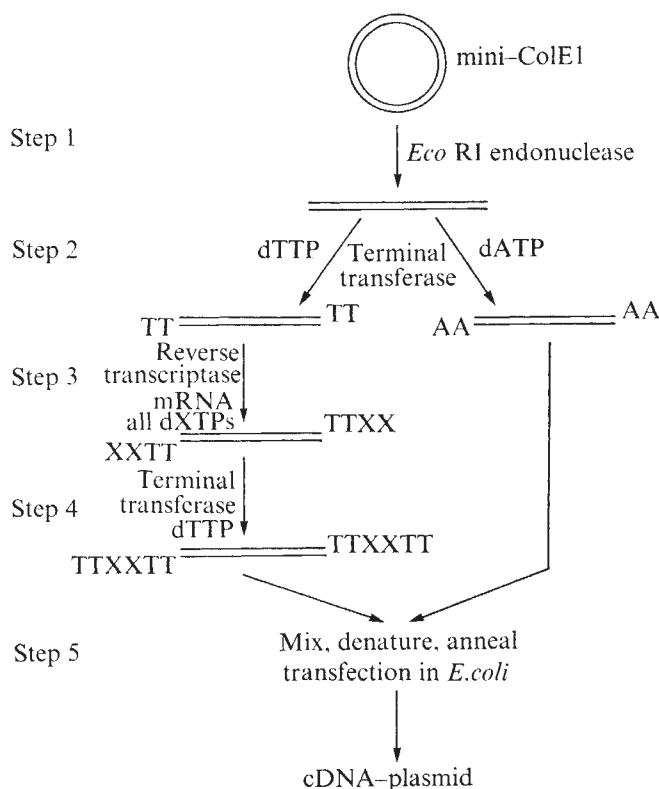


Fig. 2 General scheme for production of globin plasmids. Step one: mColE1 was prepared by the procedure described¹⁵ and *Eco*RI was prepared by the method of Yoshimori¹⁶. mColE1 DNA (20 µg) was digested with *Eco*RI (the quantity of enzyme necessary was previously determined by digestion of λ phage DNA) in 100 µl of 10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 10 mM 2-mercaptoethanol for 2 h at 37 °C. The reaction was terminated by freezing. Step two: two aliquots of 50 µl from the *Eco*RI digestion mix (10 µg DNA) were added to 50 µl containing 100 mM potassium cacodylate (pH 7), 7.5 mM potassium phosphate (pH 7), 8 mM MgCl₂, 2 mM 2-mercaptoethanol. dTTP was added to one aliquot and dATP to the other to give 0.3 mM. The tube containing dTTP also contained 10 µCi ³H-dTTP (New England Nuclear) so that the labelled plasmid DNA could easily be monitored in subsequent steps. 10 µg terminal transferase were added to each mixture and the tubes were incubated at 37 °C for 1 h. Again the mixes were frozen

to terminate the reaction. At this stage, an analytical Agarose gel was routinely carried out to check the efficiency of the transferase reaction. Step three: the mColE1 tailed with poly(dT), prepared in step 2 (10 µg DNA), was incubated in a 300-µl volume containing 20 µg rabbit globin mRNA (prepared by oligo(dT) chromatography of rabbit reticulocyte polysomal RNA¹⁷), 5 µg actinomycin D, 10 µCi ³H-dCTP (New England Nuclear, 28 Ci mmol⁻¹), 500 µM dATP, dCTP, dGTP and dTTP in the same buffer as described in the legend to Fig. 1b. Reverse transcriptase (1 µg) was added and the mixture incubated at 37 °C for 2 h. The mix was further incubated with 10 µg pancreatic ribonuclease (previously boiled to remove contaminating deoxyribonuclease activity) for 30 min at 37 °C, extracted once with an equal volume of phenol and passed over a small column of Sephadex SP-50 (in a 1-ml plastic pipette) in 0.3 M NaCl, 10 mM sodium acetate (pH 5). One-drop fractions were collected and the position of the DNA monitored by scintillation counting of 1-µl aliquots. The peak fractions were pooled and precipitated overnight at -20 °C by the addition of two volumes of ethanol. (Note: XTP refers to equal quantities of each nucleotide triphosphate.) Step four: the ethanol-precipitated mColE1 containing globin DNA sequences (prepared in step 3) was spun down, dried *in vacuo* and dissolved in 50 µl of 100 mM potassium cacodylate (pH 7), 7.5 mM potassium phosphate (pH 7), 8 mM MgCl₂, 2 mM 2-mercaptoethanol and 0.3 mM dTTP. Terminal transferase (6 µg) was added and the mix incubated at 37 °C for 1 h. Step five: after 1 h incubation in step 4, 50 µl (10 µg) of mColE1 tailed with poly(dA) (prepared in step 2) was added to the reaction mixture and the volume was increased to 250 µl with the salt concentration adjusted to 100 mM NaCl, 10 mM Tris-HCl (pH 8), 10 mM EDTA. The solution was boiled for 5 min and incubated at 70 °C for 30 min, after which 2.75 ml of the above buffer were added and incubation continued for 5.5 h at 37 °C. Finally the solution was extracted once with an equal volume of phenol and precipitated overnight at -20 °C by the addition of 2 volumes of ethanol in the presence of 50 µg *E. coli* tRNA carrier. The final dried pellet was dissolved in 300 µl of 30 mM CaCl₂, 10 mM NaCl, 0.5 mM EDTA, 10 mM Tris-HCl (pH 8) (transfection buffer).

Competent cells were prepared (as described by Cohen *et al.*¹⁸) from an overnight culture of *E. coli* C600 r⁻ m⁻ (a gift from Dr Noreen Murray). Approximately 1.2 × 10⁸ competent cells were pelleted and suspended in 300 µl of solution containing the annealed mColE1 population. (Control transfections consisted of 1 µg native mColE1 in 300 µl transfection buffer or 300 µl transfection buffer with no DNA.) The bacterial suspension was held on ice for 60 min, heated at 42 °C for 2 min and held on ice again for 60 min (with an occasional shake). 0.7 ml 2 × TY medium (1.6% (w/v) tryptone, 1% (w/v) yeast extract 0.5% (w/v) NaCl pH 7.4) was added and the bacteria incubated at 37 °C for 45 min. Sufficient colicin was added to kill 1.2 × 10⁸ cells (the dosage was previously determined) and the suspension incubated for a further 30 min at 37 °C. The cells were then pelleted, resuspended in 0.3 ml 2 × TY medium containing half the above quantity of colicin and plated on nutrient agar plates overnight at 37 °C.

should be resistant. Colicin treatment of bacteria, however, does not result in the full extinction of all non-plasmid containing bacteria and further, the annealing procedure will lead to the presence of plasmids tailed only with poly(dA) and poly(dT). The identification of clones carrying globin plasmids was facilitated by hybridisation as follows. Sixty-four randomly chosen colicin-resistant clones were grown in 1-ml cultures. Eight separate pools of eight clones were made and these pools were grown overnight in 100-ml cultures. DNA was prepared from these cultures and tested for the presence of globin plasmids by hybridisation with globin ³H-cDNA (prepared by normal reverse transcription). Table 1a shows the results of this initial screening: of the eight pools tested, half showed the ability to hybridise ³H-cDNA and thus the presence of globin plasmids. The percentage of cDNA hybridised in each case was around 30–40% of the total cDNA recovered. Since the ³H-cDNA used to assay for positive clones was a mixture of α- and β-globin sequences, 40% hybridisation approaches the proportion of the ³H-cDNA representing the α- or β-globin sequence. Identification of individual positive clones was carried out simply by growing the eight individual clones of any positive pool in 100-ml cultures, preparing DNA from these cultures and identifying the positive clone(s) by cDNA hybridisation. For example, Table 1b shows the identification of one clone containing a globin plasmid from the eight pooled clones of the initial culture number C. It seemed that in this pooled culture C,

only one clone actually contained a globin plasmid; thus, about 7% of the clones resistant to colicin actually contain globin plasmids. Most of the remainder of colicin-resistant clones (amounting to about 10 times as many clones as in a mock-transfection) are presumably due to plasmids formed from the reannealing of strands tailed with poly(dA) and poly(dT) but without added globin sequences.

The globin plasmid identified above was used in further characterisation studies. To determine whether the globin plasmid contained sequences for α or β globin, plasmid DNA was hybridised with either ³H-cDNA prepared from the original mRNA (a mixture of α- and β-globin sequences) or with ³H-cDNA prepared from mRNA originating from the rabbit reticulocyte post-ribosomal supernatant (this mRNA is enriched in α-globin mRNA²⁰). The total cDNA population was found to hybridise with the globin plasmid to around 47% whereas the α globin enriched cDNA hybridised only about 22% (Table 2). Since β-globin sequences probably predominate in the total mRNA population but are relatively depleted in the α-enriched mRNA, the DNA in the plasmid seems to be the complement of β-globin mRNA. Furthermore, when annealed with unmodified mColE1, ³H-cDNA of total globin and α-enriched globin only bound about 9% and 4% respectively to the hydroxyapatite (Table 2). This percentage is the amount of ³H-cDNA consistently found to bind to hydroxyapatite in the absence of hybridisation. Thus there are no sequences in

Table 1 Identification of clones containing globin plasmids

	Culture no.	*c.p.m. hybridised	†Recovered counts in hybrid (%)
Initial pools of eight clones <i>a</i>	<i>A</i>	5	5
	<i>B</i>	0	2
	<i>C</i>	618	43
	<i>D</i>	400	30
	<i>E</i>	500	35
	<i>F</i>	529	37
	<i>G</i>	0	1
	<i>H</i>	36	4
Individual clones of culture <i>C</i> <i>b</i>	1	0	0.4
	2	0	0
	3	2,923	31
	4	50	5
	5	6	0
	6	0	0
	7	0	0
	8	0	0

*c.p.m. hybridised = c.p.m. in 0.4 M sodium phosphate fraction (after hydroxyapatite fractionation) minus c.p.m. in 0.4-M fraction of the control tube containing 100 µg mColE1 DNA.

† % of recovered c.p.m. in 0.4-M fraction minus % c.p.m. in 0.4-M fraction of control tube.

a, After overnight growth of transfectant *E. coli* on nutrient agar plates in the presence of colicin (see legend to Fig. 2), 64 colonies were randomly selected for growth in 1 ml of 2 × TY broth. Eight pools of eight 1-ml cultures were made in 100 ml 2 × TY, grown to A550 = 0.9 and chloramphenicol added to a concentration of 200 µg ml⁻¹. Growth was continued overnight at 37 °C. The cells were collected and cleared lysates prepared as described¹⁵. DNA was precipitated for 1.5 h at 4 °C after addition of NaCl to give 750 mM and polyethylene glycol 6,000 to give 15 % (w/v). The precipitates were drained, dissolved in 1 ml 50 mM NaCl and digested at 37 °C for 30 min with 10 µg pancreatic ribonuclease; 5 µg proteinase K (Merck) was added to each tube and digestion continued for 30 min at 37 °C. At the end of this time, 10 µl 10% SDS were added, plus 1 ml 2 M NaCl, 10 mM Tris-HCl (pH 8). The DNA was extracted 3 times with equal volumes of phenol and chloroform (1:1 ratio). The final aqueous phase was sonicated for 2 × 30 s and the DNA precipitated by the addition of two volumes of ethanol (−20 °C for 2 h). The precipitates were dried and dissolved in 0.2 ml water, placed in a small siliconised tube with 2,000 c.p.m. ³H-globin cDNA (prepared by conventional oligo(dT) priming of mRNA as in ref. 19; s. a. 6 × 10⁶ cm⁻¹ µg⁻¹) and sodium phosphate buffer (pH 6.8) to give 0.24 M (total volume 0.3 ml). These mixtures were boiled for 5 min and incubated for 15 h at 70 °C. The hybridisation was assayed by hydroxyapatite fractionation as described¹⁹. A control hybridisation tube containing 100 µg sonicated mColE1 DNA and ³H-cDNA was processed to determine background binding of ³H-cDNA in 0.4 M phosphate on hydroxyapatite fractionation. *b*, The individual clones of culture *C* were grown in 100 ml culture as described for *a*. DNA preparation and hybridisation (against 10,000 c.p.m. ³H-cDNA) were also as in (*a*).

mColE1 DNA which hybridise with ³H-globin cDNA.

The globin plasmid described will contain tracts of poly(dA) and poly(dT). Therefore, it was necessary to eliminate the possibility that the hybridisation experiments were detecting hybrid formation between the poly(dA) of the plasmid and the poly(dT) tract known to be present at the end of ³H-cDNA¹⁹. Two control hybridisation experiments were carried out. The globin-plasmid DNA was annealed with mouse immunoglobulin light-chain ³H-cDNA. This cDNA will contain only poly(dT) tracts in common with sequences present in the globin plasmid. Table 2, line 6 shows that 9.6% of this cDNA binds to the hydroxyapatite after annealing with the globin plasmid compared to 5.6% binding after annealing with unmodified mColE1 DNA. Thus the poly(dT) tracts of the ³H-cDNA do not seem to be involved in hybridisation described. Furthermore, the ability of ³H-globin cDNA to hybridise with the globin plasmid in the presence of tenfold excess of unlabelled poly(dT) competitor was examined. Table 2, line 2, shows that about 45% hybridisation occurs in the presence of poly(dT), therefore the hybridisation of the ³H-cDNA is essentially unaffected by the presence of the poly(dT). This observation confirms that the hybridisation observed was due to DNA sequences complementary to globin mRNA present in the plasmid. The length of the globin segments in the plasmids is as yet unknown. It is likely, however, that the population as a whole shows a similar heterogeneity to the cDNA population made by conventional reverse transcriptase copying of mRNA. Thus an individual plasmid may contain a sequence anywhere from 50 residues to the entire length of the globin mRNA.

This paper describes the insertion of DNA copies of rabbit globin mRNA into the plasmid mColE1 and subsequent amplification by cloning in *E. coli*. These DNA copies are probably partial copies of the globin mRNA and represent the successful synthesis of part of the globin gene. A similar synthesis has also been described elsewhere⁸. The method should be applicable to any poly(A)-containing RNA or RNA to which poly(A) can be added *in vitro*, so that a large supply of the sequence can be made available. It is hoped that the ability to clone globin sequences

in bacteria will facilitate isolation, by hybridisation procedures, of those regions from the chromosomal DNA involved in control of globin gene activity. For example, the plasmid DNA could be covalently linked to cellulose to provide a basis for hybridisation/chromatographic purification of the complementary regions from nuclear DNA. The principal advantages of this method are the large quantity of DNA available, plus the important point that the plasmid contains double-stranded DNA which will allow purification of both strands of the chromosomal DNA in a single step.

In addition, manufacture of double-stranded copies of messenger RNA in mColE1 will make sequencing of such

Table 2 Hybridisation properties of purified globin plasmid

	DNA hybridised	³ H-cDNA type	c.p.m. hybridised (%)
(1)	Globin plasmid	Total globin	49.2
(2)	Globin plasmid	Total globin (in presence of poly(dT))	45.1
(3)	mColE1	Total globin	9.8
(4)	Globin plasmid	α-Enriched globin	22.7
(5)	mColE1	α-Enriched globin	3.9
(6)	Globin plasmid	Mouse Ig	9.6
(7)	mColE1	Mouse Ig	5.6

Globin plasmid DNA was prepared as described for the mColE1 DNA (Table 1). All DNA stocks were sonicated for 1 min before use. ³H-cDNA was prepared from rabbit reticulocyte 9S mRNA (total globin cDNA) from rabbit reticulocyte post-ribosomal 9S mRNA (α-enriched cDNA) and from mouse myeloma MOPC21 light chain mRNA (mouse Ig cDNA) as described¹⁹. In each hybridisation tube, 2 µg of DNA were annealed with 10,000 c.p.m. ³H-cDNA for 16 h at 70 °C in 300 µl 0.24 M sodium phosphate buffer (pH 6.8) after boiling for 5 min (Tube 2 included 10 µg poly(dT) (P.L. Biochemicals)). After annealing, the mixes were diluted with 3 ml 0.12 M phosphate containing 100 µg *Micrococcus* DNA and fractionated on 0.5 gm hydroxyapatite (BioRad HTP) as described¹⁹. % c.p.m. hybridised represents the percentage of the total recovered counts in the 0.4-M fraction from the hydroxyapatite column.

species easier by application of the rapid sequencing methods now emerging²¹. In fact, it is conceivable to clone any available RNA species (for example, eukaryotic ribosomal RNAs) by first adding poly(A) to the RNA^{22,23} and then inserting DNA copies in mColE1: the inserted DNA copies would be more amenable to sequence studies than the native RNA itself.

I thank Dr J. W. Beard for supplying reverse transcriptase, Dr C. Milstein for discussion and Mr A. Forster for technical assistance.

Addendum

Although the experiments described in this paper were, in the main, carried out before publication of the US National Institutes of Health Committee report on containing experiments with recombinant DNA, the guidelines suggest that the containment facilities used for such experiments should be mentioned in publications. It thus seems relevant to report that containment equivalent to P2 level was used initially for these experiments. Classification of this particular experiment under the NIH guidelines is rather difficult but nevertheless since publication of the guidelines, work has been carried out in conditions satisfying P3 containment.

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- ¹ Morrow, J. F., *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1743-1747 (1974).
- ² Cohen, S. N., and Chang, A. C. Y., *Molec. gen. Genet.*, **134**, 133-141 (1974).
- ³ Wensink, P. C., Finnegan, D. J., Donelson, J. E., and Hogness, D. S., *Cell*, **3**, 315-325 (1974).
- ⁴ Tanaka, T., Weisblum, B., Schnös, M., and Inman, R. B., *Biochemistry*, **14**, 2064-2072 (1975).
- ⁵ Thomas, M., Cameron, J. R., and Davis, R. W., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4579-4583 (1974).
- ⁶ Kedes, L. H., Chang, A. C. Y., Housman, D., and Cohen, S. N., *Nature*, **255**, 533-538 (1975).
- ⁷ Bishop, J. O., *Cell*, **2**, 81-86 (1974).
- ⁸ Rougeon, F., Kourilsky, P., and Mach, B., *Nucleic Acids Res.*, **2**, 2365-2378 (1975).
- ⁹ Hersfield, V., Boyer, H. W., Yanofsky, C., Lovett, M. A., and Helinski, D. R., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3455-3459 (1974).
- ¹⁰ Jackson, D. A., Symons, R. H., and Berg, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2904-2909 (1972).
- ¹¹ Jensen, R. H., Wodzinski, R. J., and Rogoff, M. H., *Biochem. biophys. Res. Commun.*, **43**, 384-392 (1971).
- ¹² Lobban, P. E., and Kaiser, A. D., *J. molec. Biol.*, **78**, 453-471 (1973).
- ¹³ Verma, I. M., Temple, G. F., Fan, H., and Baltimore, D., *Nature new Biol.*, **235**, 163-167 (1972).
- ¹⁴ Dugaiczky, A., Boyer, H. W., and Goodman, H. M., *J. molec. Biol.*, **96**, 171-184 (1975).
- ¹⁵ Clewell, D. B., and Helinski, D. R., *Biochemistry*, **9**, 4428-4440 (1970).
- ¹⁶ Yoshimori, R. N., thesis, Univ. California (1971).
- ¹⁷ Aviv, H., and Leder, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1408-1412 (1972).
- ¹⁸ Cohen, S. N., Chang, A. C. Y., and Hsu, L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2110-2114 (1972).
- ¹⁹ Rabbitts, T. H., and Milstein, C., *Eur. J. Biochem.*, **52**, 125-133 (1975).
- ²⁰ Jacobs-Lorena, M., and Baglioni, C., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1425-1428 (1972).
- ²¹ Sanger, F., and Coulson, A. F., *J. molec. Biol.*, **94**, 441-448 (1975).
- ²² Getz, M. J., Birnie, G. D., and Paul, J., *Biochemistry*, **13**, 2235-2240 (1974).
- ²³ Thrall, C. J., *et al.*, *Biochem. biophys. Res. Commun.*, **61**, 1443-1449 (1974).

letters to nature

Annihilation of matter and antimatter and the cosmic X-ray background

In cosmological models where matter and antimatter occur symmetrically in the Universe¹⁻⁵, their annihilation is likely to take place also at the present time. At the annihilation of a proton and an antiproton, ~ 1.6 negative electrons and an equal number of positrons, each with an energy of ~ 10 -100 MeV are produced (Fig. 1), together with γ rays and neutrinos⁶. The initial electron energies can be expected to decrease gradually because of their interaction with cosmic magnetic fields, plasmas, and electromagnetic radiation. As a result of these interactions synchrotron radiation, bremsstrahlung, and

inverse Compton radiation are all emitted. When optical photons are scattered against the relativistic electrons, X rays are produced by the inverse Compton effect. This mechanism may be an important source of cosmic X rays. Our purpose here is to study how efficient this X-ray mechanism can be in intergalactic space (where most of the annihilation electrons are expected to be found) and to see whether it can offer an explanation to the cosmic X-ray background observed (see refs 7-9).

For the sake of clarity we shall consider a simplified model of the X-ray mechanism assuming:

- (1) That the annihilation of protons and antiprotons occurs at a constant rate giving rise to relativistic electrons with a production spectrum according to Fig. 1, with a maximum close to 30 MeV.
- (2) That the energy of the electrons decreases continuously, primarily from synchrotron losses, so that a stationary energy spectrum is obtained for the electrons.
- (3) That the primary optical radiation in intergalactic space (with which the annihilation electrons interact and produce X rays) consists mainly of galactic light having an intensity of $S_{10} = 1.4$ ($m_v = 10$) star degree⁻² (ref. 10) and a spectrum with the shape of a black-body distribution at a temperature $T_{ph} = 6 \times 10^3$ K.

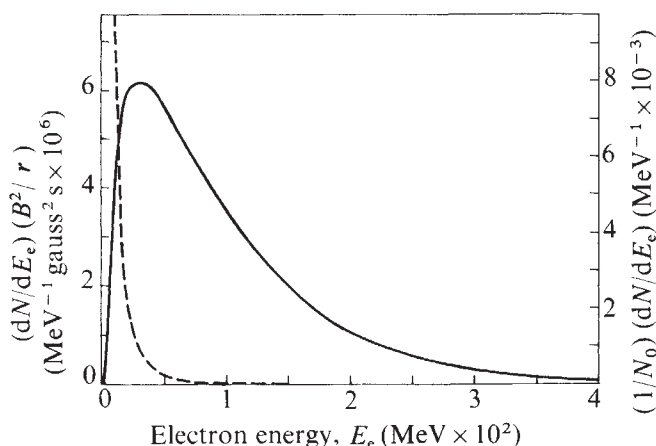
For assumptions (1) and (2), Ekspeng *et al.*⁶ have estimated the stationary energy spectrum of the annihilation electrons (Fig. 1). A considerable concentration of electrons towards small energies can be noticed in the graph. It should be mentioned that the shape of this spectrum is unchanged if one includes energy losses from inverse Compton scattering of the electrons against black-body radiation of low energy.

Korshak¹¹ has calculated the spectrum of the inverse Compton radiation produced by one electron of energy E_e interacting with black-body photons of temperature T_{ph} and density n_{ph} . He finds

$$P_C(\nu) = 1.24 \sigma_T c n_{ph} F(\nu/\nu_c) \text{ erg s}^{-1} \text{ Hz}^{-1} \quad (1)$$

provided $E_e \gg m_e c^2$, $\beta E_e \gg h\nu \gg kT_{ph}/\beta$, ($\beta = v/c \simeq 1$).

Fig. 1 —, Energy spectrum of electrons (negative and positive) directly-produced by proton-antiproton annihilation. The spectrum is normalised by means of the total number of electrons N_0 formed. —, Stationary energy spectrum of annihilation electrons dN/dE_e multiplied by B^2/r . The spectrum is obtained for a constant rate of annihilation r when the electrons lose energy to synchrotron radiation in a magnetic field B .



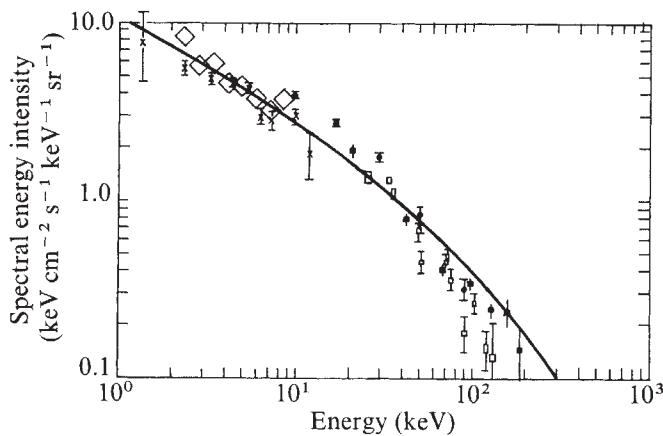


Fig. 2 Spectrum of inverse Compton radiation resulting from scattering of galactic light on annihilation electrons which decrease in energy because of synchrotron radiation (solid curve). The inverse Compton spectrum is compared with measured intensities of the cosmic X-ray background in the energy interval $1 \text{ keV} \lesssim h\nu \lesssim 10^2 \text{ keV}$ (ref. 9). The experimental points are from: ●, OSO III; ◇, LLL; ×, ASE; □, Leiden-Nagoya; ■, GSFC-OSO V.

$\sigma_T = 6.65 \times 10^{-25} \text{ cm}^2$ is the Thomson cross section, ν is the frequency of the scattered photons, and

$$\nu_c = (4kT_{ph}/h) (E_e/m_e c^2)^2.$$

The function $F(\nu/\nu_c)$ reaches its maximum value of 0.254 at $\nu \approx \nu_c$ and is $\approx 1.65\nu/\nu_c$ for $\nu/\nu_c \ll 1$ and

$$\approx (\nu/\nu_c) \exp(-\nu/\nu_c) \text{ for } \nu/\nu_c \gg 1$$

By integrating numerically the spectrum defined by equation (1) over the stationary energy distribution of the relativistic electrons (dashed curve in Fig. 1) we can estimate the spectrum of the inverse Compton radiation in our model, which is shown in Fig. 2, together with the measured intensities of the cosmic X-ray background in the interval $1 \text{ keV} \lesssim h\nu \lesssim 10^2 \text{ keV}$ (ref. 9). As is clear from Fig. 2, the inverse Compton spectrum agrees well with the observed spectrum in this interval.

It should be noted that except for the total intensity there is no adjustable parameter in this derivation. For photon energies $> 1 \text{ MeV}$, the inverse Compton spectrum starts to deviate appreciably from observational data⁷. The maximum photon energy of the inverse Compton radiation that can be produced by our mechanism is well above 10 MeV .

The intensity of the inverse Compton radiation is proportional to the density of the primary photons and to the density of the relativistic electrons. Furthermore, the intensity depends also on the size of the volume which the electrons occupy. With the radius of the volume being $R = 10^{10} \text{ light yr} = 10^{28} \text{ cm}$ and the primary radiation consisting of galactic light as assumed in (2), the intensity in Fig. 2 is obtained for a mean electron density $n_e \approx 10^{-9} \text{ cm}^{-3}$ in the energy interval $10 \text{ MeV} < E_e < 10^2 \text{ MeV}$. This electron density is about two orders of magnitude smaller than the mean atom density of galactic material throughout the Universe.

The electrons which are necessary to produce the cosmic X-ray background will of course also give rise to other types of electromagnetic radiation. One of these is the synchrotron radiation whose intensity and frequency is influenced by the magnetic field B . To obtain a stationary spectrum for the electron energies of interest, $E_e \gtrsim 1 \text{ MeV}$ (as assumed in (2)), the magnetic field must be so strong that the decay time (τ_s) arising from synchrotron losses

$$\tau_s = \frac{5.1 \times 10^8}{B^2} \frac{1}{1 + E_e/m_e c^2} \text{ s} \quad (2)$$

(B in gauss) is at least less than the age of the Universe, being $\sim 10^{10} \text{ yr} = 3 \times 10^{17} \text{ s}$. Thus, the magnetic field must be $\gtrsim 2 \times 10^{-8} \text{ gauss}$.

With a magnetic field of this strength, the intensity of synchrotron radiation in the radio band produced by the annihilation electrons necessary to generate the X rays, would be higher than experiment allows¹²⁻¹⁴. The solution of this problem may be that the annihilation electrons are created locally in quasars and galactic nuclei¹⁵, where the magnetic field is sufficiently strong to cause a rapid decay in the energy of the electrons. This could also lead to absorption of the γ rays formed by annihilation, so that their flux does not become unacceptably high^{16,17}. The annihilation electrons, on the other hand, escape into intergalactic space where they become uniformly distributed and where they produce the X-ray background by interacting with optical photons.

We have for simplicity disregarded effects of cosmological evolution, but, nevertheless, our description may be adequate in a framework such as the Klein-Alfvén metagalactic theory. A more detailed discussion has to wait for general development of the symmetric cosmology.

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PER CARLQVIST

*Department of Plasma Physics,
Royal Institute of Technology,
S-100 44 Stockholm 70*

BERTEL LAURENT

*Institute of Theoretical Physics,
University of Stockholm,
S-113 46 Stockholm, Sweden*

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- ¹ Klein, O., *Mém. Soc. Sci. Liège, Quatrième Serie*, XIII, 42-51 (1953).
- ² Klein, O., *Nature*, **211**, 1337-1341 (1966).
- ³ Alfvén, H., and Klein, O., *Arkiv för Fysik*, **23**, 187-194 (1963).
- ⁴ Alfvén, H., *Rev. Mod. Phys.*, **37**, 652-665 (1965).
- ⁵ Omnes, R. L., *Phys. Rev. Lett.*, **23**, 38-40 (1969).
- ⁶ Ekspöng, A. G., Yamadagni, N. K., Bonnevier, B., *Phys. Rev. Lett.*, **16**, 664-667 (1966).
- ⁷ Kasturirangan, K., and Rao, U. R., *Astrophys. Space Sci.*, **15**, 161-166 (1972).
- ⁸ Adams, D. J., and Ricketts, M. J., *Astrophys. Space Sci.*, **24**, 585-592 (1973).
- ⁹ Schwartz, D. A., in *Int. Conf. X-Rays in Space, Calgary, Alberta, August 14-21, 1974*, 1096-1119.
- ¹⁰ Allen, C. W., *Astrophysical Quantities*, 3rd ed., 289 (1973).
- ¹¹ Korchak, A. A., *Soviet Astr.*, **11**, 258-263 (1967).
- ¹² Turtle, A. J., Pugh, J. F., Kenderdine, S., and Pauliny-Toth, I. I. K., *Mon. Not. R. astr. Soc.*, **124**, 297-312 (1962).
- ¹³ Smith, F. G., *Mon. Not. R. astr. Soc.*, **131**, 145-153 (1965).
- ¹⁴ Gould, R. J., and Schröder, G. P., *Phys. Rev.*, **155**, 1408-1411 (1967).
- ¹⁵ Alfvén, H., and Elvius, A., *Science*, **164**, 911-917 (1969).
- ¹⁶ Elvius, A., *IAU Symp.*, No. 44, 306-310 (1972).
- ¹⁷ Elvius, A., Karlson, E. T., and Laurent, B. E., *IAU Symp.*, No. 63, 341-345 (1974).

X-ray observations of GX17+2 and GX9+9 by Aryabhata

X-RAY observations on GX17+2 and GX9+9 were made using the proportional counter telescope on board the first Indian satellite 'Aryabhata'. We present these results, which enable us to start discussing source mechanisms for these objects.

The proportional counter had an effective area of 15.4 cm^2 and was fitted with a 0.0635-mm beryllium entrance window. The counter was filled with a gas mixture of Ar, CO_2 and He in the pressure ratio of $0.9 : 0.095 : 0.005$ to a total pressure of 1 atm . Cylindrical aluminium collimators defined a field of view of 12.5° FWHM for the counter. The telescope was mounted with its look direction along the spin axis of the satellite. The details of the payload are being published elsewhere¹.

Observations on GX17+2 and GX9+9 were made by the proportional counter telescope in the thirty-first orbit, before the spacecraft was spin stabilised, when it was performing a slow spin around an axis different from the spin axis. Sighting of GX17+2 occurred at 110 d 8 h 9 min 8 s (UT), followed by that of GX9+9. The identifications of

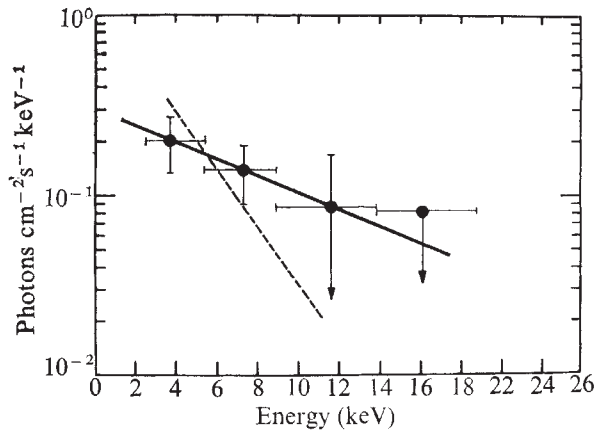
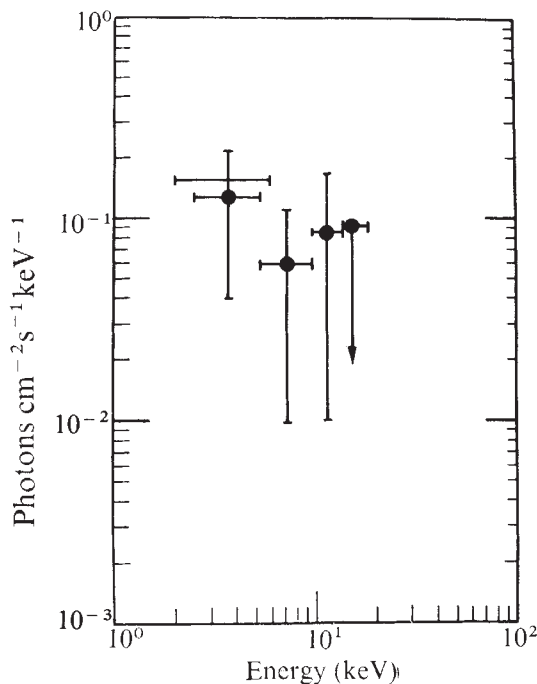


Fig. 1 Spectrum of GX17+2 obtained from Aryabhata observations. The dashed line represents the estimated temperature from the Lawrence Livermore group (42×10^6 K). The solid line represents our temperature (108×10^6 K).

GX17+2 and GX9+9 were based on the available positional data on these sources²⁻⁴. Data were obtained in four pulse height intervals over the energy range of 2.5–18.75 keV. The contributions of the two sources to the observed counting rates were derived after making allowance for the background effects as deduced from the examination of the nature of counting-rate profiles and their known geomagnetic dependence. Further, in the case of GX17+2, it was necessary to take into account contributory effects from GX+13.5 which was partially in the field of view for part of the observation time. These corrections were based on the available data⁷ on this source, and the effect so estimated was $\sim 20\%$. The photon spectra for GX17+2 and GX9+9 were derived after correcting for the efficiency of the counter and the telescopic response of the detector. The spectra so deduced for these sources are shown in Figs 1 and 2.

The spectrum of GX17+2 can be fitted to an exponential function in the present case, and yields a characteristic kT value of 9.31 ± 0.3 keV. If the X radiation arises from bremsstrahlung in a hot plasma, the equivalent temperature

Fig. 2 Spectrum of GX9+9 obtained from Aryabhata observations. —, Uhuru; ●, Aryabhata.



of the emitting body was $(108 \pm 4) \times 10^6$ K at the time of observation.

GX17+2 is one of the relatively intense X-ray sources in the Scorpius-Sagittarius region. Observations on this source⁵⁻⁷ had provided information primarily on its position rather than detailed emission features. The first set of detailed observations⁸ were made by Uhuru detectors in late 1970 and early 1971 and revealed the variable character of X-ray emission besides establishing the exponential nature of the spectrum. Rocket-borne observations by the Lawrence Livermore Laboratory (LLL) group (R. W. Hill *et al.*, unpublished) have provided confirmation of the thermal nature of its emission. It seems that the source exhibits variations over a wide range of temperatures from 25×10^6 to 155×10^6 K. A closer analysis of the temperature-emission flux relationship in the 2–7 keV energy range leads, however, to no definite correlation between these two parameters. Further, when we consider all the existing data on this source, no definite conclusion can be drawn about the existence of a two-state emission character for this source as suggested by Tananbaum *et al.*⁸. The source seems to be similar in its properties to Sco X-1 as deduced from the comparison between radio and X-ray observations⁹, but the lack of optical data on the source is a serious constraint to further analysis.

The spectral distribution of GX9+9 seems to be governed by a power law function with an exponent value of ~ 1.2 , and is suggestive of a non-thermal mechanism for X-ray emission. In the energy interval of 2.5–10 keV, the integrated energy flux is $(0.57 \pm 0.3) \times 10^8$ erg cm⁻² s⁻¹. The flux obtained by Bradt *et al.*³ in a rocket flight in 1967 was 0.25×10^{-8} erg cm⁻² s⁻¹ in the energy range 1.5–6 keV, whereas the same group⁴ reported a flux of $\sim 0.77 \times 10^{-8}$ erg cm⁻² s⁻¹ in the 1.5–8 keV energy interval from a subsequent observation in 1968. Comparison of the energy fluxes obtained from our observation and the two earlier rocket observations, after normalising to the same energy interval of 1.5–8 keV, leads to the suggestion that X-ray emission from GX9+9 is variable.

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K. KASTURIRANGAN
U. R. RAO
D. P. SHARMA
M. S. RADHA

ISRO Satellite Systems Project,
Bangalore 562140,
India

Received January 5; accepted January 30, 1976.

- ¹ Rao, U. R., *et al.*, (in the press).
- ² Giacconi, R., *et al.*, *Astrophys. J. Suppl.*, **27**, 37 (1974).
- ³ Bradt, H., *et al.*, *Astrophys. J.*, **152**, 1005 (1968).
- ⁴ Meyer, W., *et al.*, *Astrophys. J. Lett.*, **159**, L115 (1970).
- ⁵ Fisher, P., *et al.*, *Astrophys. J.*, **151**, 1 (1968).
- ⁶ Gursky, H., *et al.*, *Astrophys. J. Lett.*, **150**, L75 (1967).
- ⁷ Schnopper, H., *et al.*, *Astrophys. J. Lett.*, **161**, L161 (1970).
- ⁸ Tananbaum, H., *et al.*, *Astrophys. J. Lett.*, **168**, L28 (1971).
- ⁹ Hjellming, R. M., and Wade, C. M., *Astrophys. J.*, **168**, L21 (1971).

Reliability of sunspots as tracers of solar surface rotation

New methods of measuring solar rotation have produced significantly different rotation speeds to those derived from sunspots^{1,2}. Spectroscopic measurements of Doppler displacements give a so-called plasma rotation velocity of 13.76° d⁻¹ (sidereal) at the solar equator³ as against 14.38° d⁻¹ (sidereal) from sunspot proper motion velocity^{4,5}. Other techniques of measuring solar rotation lead to differences in the differential

Table 1 Sunspot properties and derived solar rotation velocities

	Spot SPO3703	Spot SPO3707	Spot SPO3710
Central meridian passage (UT)	Oct. 7.3 1975	Nov. 10.6 1975	Nov. 19.9 1975
Heliographic latitude	+32.5°	+3.5°	-7.0°
Number of days observed during passage	13	9	11
Proper motion rotation (synodic) (degree d ⁻¹)	12.66±0.10	13.36±0.10	13.12±0.10
Proper motion rotation (sidereal) (degree d ⁻¹)	13.65±0.10	14.35±0.10	14.11±0.10
Plasma rotation (sidereal) (degree d ⁻¹)	12.47±0.15	13.16±0.20	13.61±0.20
Velocity difference (proper motion—plasma) (m s ⁻¹)	+140±20	+170±30	+60±30

rotation from a strong variation of the solar rotation with latitude for sunspots⁴ and photospheric plasma³ to hardly any variation at all for coronal holes⁶. Our understanding of these differences is very poor. It is therefore appropriate to obtain additional accurate solar rotation measurements with as many different techniques as possible and to check the validity of the assumption that the proper motion of tracers, like sunspots, correspond to the actual motion of matter on the solar surface. We report here the result of plasma rotation velocity measurements inside sunspot umbrae and their relation to the rotation velocities derived from spot proper motions. We find that plasmas inside and outside the spots rotate at similar rates, and conclude that sunspots make poor tracers.

The spectrographic measurements consist of sequences of spectra of the sunspot umbrae in the neutral titanium line at 571.39 nm. This line has been selected because of the following favourable properties⁷: (1) it has no Zeeman splitting; this

makes it possible to locate its wavelength very precisely, independent of instrument polarisation properties; (2) it is strongly enhanced in sunspot umbrae with respect to the surrounding penumbra and photosphere; this changes in apparent lineshifts resulting from stray light from the atmosphere instrument and seeing; (3) it falls in a window between the numerous absorption lines introduced in the spectrum by an iodine absorption tube. The sunspot is imaged through this absorption tube, which is placed just in front of the spectrograph slit. The numerous weak but very sharp iodine absorption lines provide a very stable wavelength reference for the Ti I line⁸. Since the iodine absorption spectrum is observed simultaneously with the sunspot spectrum (using light originating in the spot), effects arising from different spectrograph conditions, which often limit the accuracy when using emission reference lines, are eliminated. The spectra were recorded on Kodak SO-392 film with a dispersion of 96.9 mm nm⁻¹ with an exposure time of ~ 2 min. The Sacramento Peak Observatory 16'' coronagraph was used to image the Sun, on the scale of ~ 8'' mm⁻¹.

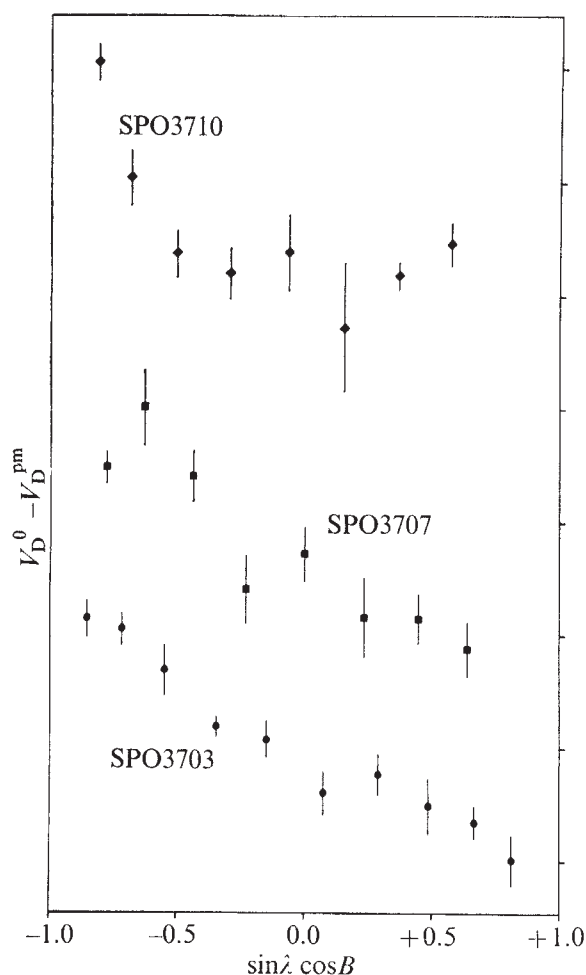
Table 1 lists the three sunspots for which rather complete coverage was obtained while the spot moved from the solar E-W limb. Two spots belong to the old cycle, one to the new. For each of the spots 60–100 spectra were obtained and analysed. The Ti I line could be located with respect to the nearby iodine lines with an accuracy 0.1 pm, corresponding to a Doppler velocity of ~ 50 m s⁻¹. Observations of the proper motion of the sunspots were made at the Sacramento Peak Observatory and at the NOAA Space Environmental Forecasting Center in Boulder. Table 1 also lists the synodic and sidereal rotation velocities derived from these proper motions. The latitude drift of the spots was very minor, ≤ 2° during the spot passage across the solar disk (or ≤ 20 m s⁻¹). After correction for the rotation and orbital velocities of the Earth the line of sight velocity V_D for a point on the Sun with heliographic longitude λ and latitude β may be shown to equal

$$V_D = V_\lambda \sin \lambda \cos B + V_\beta \cos \theta' - V_n \cos \theta \quad (1)$$

where V_D is positive for a velocity away from the observer, (V_λ , V_β , V_n) are the velocity components on the Sun in positive longitude, latitude and height directions, B is the heliographic latitude of the centre of the solar disk, θ is the angle between the observed point on the Sun and the Earth as seen from the centre of the Sun and θ' is the same as θ , but for a point in the opposite hemisphere at the same longitude, λ , but 90° away from the observed point. The last two terms in equation (1) are symmetrical around the central meridian ($\lambda = 0$), whereas the rotation term is not. This allows the determination of the rotation to be quite independent of any other velocity field. Figure 1 displays the difference in the observed Doppler velocity V_D^0 and the velocity V_D^{pm} as predicted by the spot proper motions (and equation (1)) as a function of $\sin \lambda \cos B$. It is quite obvious that the observed plasma rotation velocity is significantly less than the spot proper motion rotation velocity. Table 1 lists the resulting plasma rotation velocities (degree d⁻¹) and the velocity differences from the proper motion values (m s⁻¹).

The difference between the two rotation rates is quite surprising since it is generally believed that the magnetic fields on the Sun are 'frozen-in' the plasma so that the two rotation rates should have been the same. The difference also shows that

Fig. 1 Difference in line-of-sight velocity for observed plasma velocity (V_D^0) and as derived from the sunspot proper motion (V_D^{pm}). The zero point for V_D^0 has not yet been determined, and is therefore not indicated. One division on the ordinate corresponds to 100 m s⁻¹. Error bars correspond to 1 s.d. The curvature of the data for the SPO3710 spot is probably the result of the third term in equation (1) indicating an upflow of ~ 125 m s⁻¹ in the umbra.



spots are poor tracers for measuring solar surface motions. The faster rotation of the spots (found to be close to the average sunspot values¹) must be the results of subsurface effects, perhaps indicating an increase of rotation rate with depth. The slower plasma rotation velocities for sunspots makes the spot plasma rotate at similar rates as the plasma outside sunspots³. It should be noted here that P. Foukal (unpublished) found the solar surface plasma rotation velocity to increase with magnetic field strength in the plasma. This effect obviously does not persist for the very high field strengths found in sunspots. Future work will include comparison of spot and non-spot plasma rotation rates, analysis of the data for V_θ and V_h velocity components and observations of (V_θ , V_ϕ , V_h) for a number of sunspots within the same sunspot group.

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JACQUES MAURICE BECKERS

Sacramento Peak Observatory,
Air Force Cambridge Research Laboratories,
Sunspot, New Mexico 88349

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¹ Gilman, P. A., *A. Rev. Astr. Astrophys.*, **12**, 47 (1974).

² Beckers, J. M., and Canfield, R. C., *Proc. Nice Symp. Motions in Stellar Atmospheres* (edit. by Cayrel, R.) (in the press).

³ Howard, R., and Harvey, J. W., *Solar Physics*, **12**, 23 (1970).

⁴ Newton, H. W., and Nunn, H. L., *Mon. Not. R. astr. Soc.*, **111**, 413 (1961).

⁵ Ward, F., *Astrophys. J.*, **141**, 534 (1965).

⁶ Wagner, W. J., *Astrophys. J. Lett.*, **198**, L141 (1975).

⁷ Beckers, J. M., *Astrophys. J.*, (in the press).

⁸ Schweitzer, W. G., Jr., Kessler, E. G., Jr., Deslattes, R. D., Layer, H. P., and Whetstone, J. R., *Appl. Opt.*, **12**, 2927 (1973).

Physical argument and hypothesis for Sun-weather relationships

A GREAT deal of attention has been focused on the subject of Sun-weather relationships^{1,2}. There is a clear need for physical processes and hypotheses to be brought forward and tested in this area. I propose here two theoretical schemes to account for some of the observed correlations; one is a physical argument suggested earlier³, and expanded here, that the weather may generate electrical effects in the ionosphere (which may in turn affect the magnetopause), and the other is an hypothesis that the total emissivity of the Sun (both particles and electromagnetic radiation) remains constant. I investigate the possible consequences of this hypothesis.

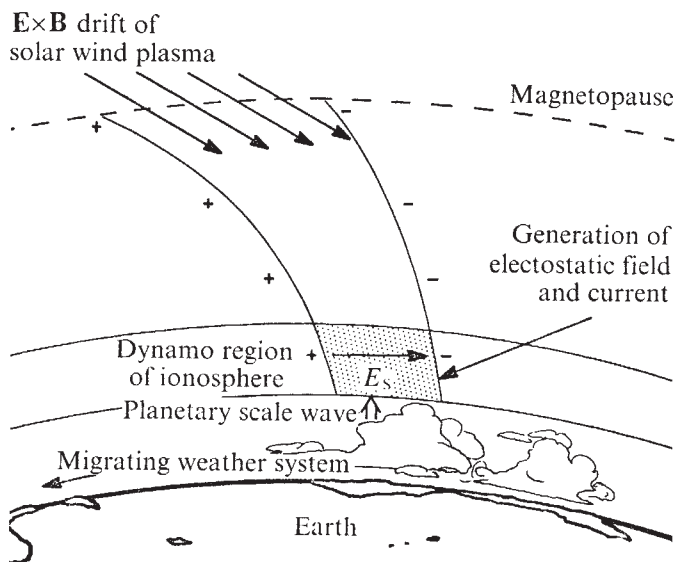
First, the physical argument. There is strong evidence that weather systems in the lower atmosphere can launch energy into the ionosphere and there generate winds on a planetary scale⁴. In the dynamo region of the ionosphere the winds can generate electric currents and therefore magnetic disturbance directly⁵. Moreover, new electrostatic fields can be generated by the wind, which can be up to one order of magnitude greater than the wind dynamo fields⁶: their magnitude can be as great as 50 mV m⁻¹ at the ionosphere. When there is good conductivity along the geomagnetic field, these fields can be mapped throughout the magnetosphere. If these new electrostatic fields occur on magnetic field lines connected to the magnetopause, solar wind plasma can be moved by the $E \times B$ drift into the magnetosphere³. The plasma so caught in the magnetosphere can be a source of energy for subsequent magnetic disturbance of direct solar wind origin. It should be unnecessary to state that there is continual interplay in this fashion between winds in the ionospheric dynamo region and the magnetopause, no matter what their origin. A field of 50 mV m⁻¹ at the ionosphere could cause a field of ~ 1.5 mV m⁻¹ at the magnetopause because of the reduction in strength of the magnetic field there. This electric field is comparable in order of magnitude with that measured

near the magnetopause by Aggson (see ref. 6). To drift solar wind plasma across the magnetopause one must have a component of electric field perpendicular to B and parallel to the magnetopause. Even if the angle between the electric field transmitted from the ionosphere and the normal to the magnetopause were as high as 85°, the drift speed of solar wind plasma across the magnetopause (where the magnetic field is assumed to be of strength ~ 50 nT) would still be 2.6 km s⁻¹. Given a solar wind⁷ of density 10 cm⁻³ and speed of 300 km s⁻¹ and an area of capture of (30 Earth radii)², an input of energy to the magnetosphere of 7×10^{17} erg s⁻¹ would be produced. This is comparable with the consumption of energy in the thermosphere from moderate magnetic disturbance^{8,9}.

In giving a physical interpretation to the correlations between magnetic disturbance and the weather in the lower atmosphere¹⁰, one must further understand the migration path and the time constant of the weather systems. The complex wave communication time from the lower atmosphere to the very magnetopause may only be of the order of hours, but the time constant of weather systems of the order of days. It is entirely possible, then, that a primitive weather system while in polar regions could initiate magnetic disturbance in the above manner (see Fig. 1). This system could then migrate to middle latitude where it would seem to have followed, rather than initiated, the magnetic disturbance. This does not rule out the possibility that some (at this stage unknown) mechanism may allow magnetic disturbance to initiate some weather systems; on the other hand, the physical system propounded here could provide feedback to such an unknown mechanism. To test the foregoing theory, studies of correlations of magnetic disturbance and polar weather systems on or near magnetic field lines that connect to the magnetopause¹¹ should be performed.

We now present a new hypothesis. Note first that the solar 'constant' and the energy of emission of the solar wind near the plane of the ecliptic are similar when expressed in mass units through Einstein's well known relationship $E = mc^2$. The solar constant is¹² ~ 1.9 calorie min⁻¹ cm⁻² $\sim 1.48 \times 10^{-15}$ gm cm⁻² s⁻¹. The mean solar wind near the plane of the ecliptic at 1 AU from the sun has a density of 8 proton-electron pairs cm⁻³ and a radial speed of 320 km s⁻¹. This represents a mass flux of 4.3×10^{-16} gm cm⁻² s⁻¹. The kinetic energies of the protons and electrons are not included in this estimate because they make only a small contribution. Of course helium and more massive ions may make up a substantial contribution to the mass flux also⁷. If the number density of helium reaches 25%, its mass flux then equals that of protons. A solar wind with ten proton-electron pairs per cm³ and two helium ions per cm³ and

Fig. 1 Modulation of solar wind capture by the weather



a speed of 633 km s^{-1} would equal, in mass flux, the solar 'constant'. It is possible that there is interplay between these two energy components at some fundamental level in the Sun.

Now the total energy (S) of emission of the sun may be written in mass units as

$$S = \int_0^{2\pi} \int_0^\pi \left[\int_0^\infty \frac{\varepsilon(\lambda) d\lambda}{c^2} + \sum n_s m_s v + \frac{v}{8\pi c^2} [B^2 + E^2 + K] \right] \times \\ \times R^2 \sin\theta d\theta d\phi$$

where $\varepsilon(\lambda)$ is the electromagnetic energy flux Å^{-1} ; n_s , m_s , v the number density, mass and radial speed of a species s of particle; B and E the d.c. magnetic and electric field connected with the solar wind; K is the kinetic energy density associated with the thermal and organised motions of the solar wind particles; and R is radial distance from the centre of the Sun.

The most important of the terms of S in interplanetary space are those involving $\varepsilon(\lambda)$ and n_s , m_s , v . The theory of the solar wind¹³ demonstrates the interaction of the parameters n_s , v and K in the lower reaches of the solar corona, where thermal energy density is converted to kinetic energy of expansion. There may be interplay between the terms involving $\varepsilon(\lambda)$ and those involving n_s , m_s and v at some levels in the Sun's outer layers perhaps somewhere in the chromosphere. I hypothesise that variability in S may be considerably less than variability of the component terms of S . The test of the possible constancy of the fundamental parameter S should be an added reason for an 'out-of-the-ecliptic-mission'.

This hypothesis has application to the modulation of the solar corona and to Sun-weather relationships and in particular to that involving interplanetary magnetic field sector structure¹⁴.

On the first of these questions we note that extreme ultraviolet emission from the Sun may modulate the solar wind speed, as indicated below. In passing we note that the kinetic energy density flux of the solar wind ($\frac{1}{2} n_s m_s v^3$) is of the same order of magnitude as the combined extreme ultraviolet and ultraviolet flux at 1 AU from the Sun, that is, in the range $0.1\text{--}1 \text{ erg cm}^{-2} \text{ s}^{-1}$ (refs 7 and 15). There may be coupling between these fluxes. Indeed, recent X-ray images of the Sun show a relationship in space between depressions of X-ray intensity in the 3–32 Å and 44–54 Å band and increases in solar wind speed. Moreover, the depressions in X-ray intensities are related spatially to magnetic field structures on the Sun, being located between lines of polarity reversals¹⁶. We also note that magnetic structure in interplanetary space is mappable, to a good degree, with present knowledge, back to the Sun¹⁷. I suggest that the correlation of some weather systems and interplanetary magnetic field structure is traceable in physical origin back to the physical processes involved in the interplay of energy of solar wind at its origin, the magnetic field there, and the accompanying electromagnetic radiation. In terms of the hypothesis about S , this would mean that the weather modification associated with interplanetary sector structure is caused by a modulation of electromagnetic radiation (not necessarily restricted to the XUV range) emission from the outer layers of the Sun in specific regions in which the solar wind velocity and magnetic field structure are also altered. The possible occurrence of geomagnetic disturbance at an intermediate stage may be incidental and the weather modification can be traced more directly to the modulation of electromagnetic radiation at the Sun. Since it is now feasible technologically to look at a wide range of solar emissions with satellites, measurement of the comprehensive parameter S should be made, because it may be more basic to the understanding of Sun-weather relationships than the conventional solar constant. In the meantime it would be fruitful to search for a correlation in time of the conventional solar 'constant' and interplanetary sector structure. It should be noted that a

correlation of time variations of intensities in the ultraviolet part of the solar spectrum with sector boundary crossings has already been noted¹⁸.

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K. D. COLE

Division of Theoretical and Space Physics,
La Trobe University,
Bundoora, Victoria 3083, Australia

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- ¹ *Proc. Symp. on Possible Relationships Between Solar Activity and Meteorological Phenomena* (edit. by Bandeen, W. R., and Maran, S. P.) (NASA/Goddard Space Flight Center, Greenbelt, Maryland, 1970).
- ² King, J. W., *Astronautics and Aeronautics*, 10 (April 1975).
- ³ Cole, K. D., *Planet. Space Sci.*, 22, 1075 (1974).
- ⁴ Beynon, W. J. G., and Winstanley, E. H., *Nature*, 222, 1262 (1969).
- ⁵ Cole, K. D., *Aust. J. Phys.*, 13, 484 (1960).
- ⁶ Heppner, J. P., *Proc. Symp. on Critical Problems of Magnetospheric Physics*, 107 (IUCSTP Secretariat, Washington, DC, 1970).
- ⁷ Hundhausen, A. J., *Rev. Geophys.*, 8, 729 (1970).
- ⁸ Cole, K. D., *Aust. J. Phys.*, 15, 223 (1962).
- ⁹ Cole, K. D., *Planet. Space Sci.*, 1, 000, 1971.
- ¹⁰ Roberts, W. O., and Olson, R. H., *J. Atmos. Sci.*, 30, 135 (1973).
- ¹¹ Fairfield, D. H., *J. geophys. Res.*, 23, 7329 (1968).
- ¹² Fritz, S., in *Compendium of Meteorology* (edit. by Malone, T. F.), 18 (American Meteorological Society, Boston, Massachusetts, 1951).
- ¹³ Parker, E. N., *Space Sci. Rev.*, 4, 666 (1965).
- ¹⁴ Wilcox, J. M., *J. Atmos. Sci.*, 31, 581 (1974).
- ¹⁵ Hinteregger, H. E., Hale, L. A., and Schmidtke, G. S., *Space Sci. Rev.*, 5, 1175 (1965).
- ¹⁶ McIntosh, P. S., *et al.*, *EOS, Trans. Am. geophys. Un.*, 56, 438 (1975).
- ¹⁷ Schatten, K. H., Wilcox, J. M., and Ness, N. F., *Solar Phys.*, 6, 442 (1969).
- ¹⁸ Heath, D. F., and Wilcox, J. M., in *Proc. Symp. on Possible Relationship Between Solar Activity and Meteorological Phenomena* (edit. by Bandeen, W. R., and Maran, S. P.) (NASA/Goddard Space Flight Center, Greenbelt, Maryland, 1970).

Early lunar magnetism

We have applied a new method¹ for palaeointensity (ancient magnetic field) to three subsamples of a single, 1-m homogeneous clast (72215) from a recrystallised boulder of lunar breccia. Samples from the clast have been dated by ⁴⁰Ar–³⁹Ar (ref. 2), Rb–Sr (ref. 3) and U–Pb (ref. 4) methods to yield a date of 4.0×10^9 yr as the age of boulder assembly. The new method¹ has two desirable characteristics; first, its reliance on simulating the natural remanent magnetisation (NRM) directly with a thermoremanent magnetisation (TRM); second, it includes a built-in monitoring device which detects thermally-induced sample degradation (oxidation, sintering and annealing), a common occurrence in lunar samples. We therefore provide here the first credible evidence that the strength of the ambient magnetic field at the Taurus–Littrow region of the Moon must have been about 0.4 oersted, 4.0×10^9 yr BP.

Of the two main models for the origin of the lunar magnetic field—the iron-core dynamo⁵ and the uniformly magnetised lunar silicate core^{6,7}—the former more easily explains our observation of an average magnetic field of 0.4 oersted during early lunar history. But in view of the similarity between the size of this field and that deduced^{8–10} from carbonaceous chondrites 4.4×10^9 yr old, we draw attention to a relatively little considered possibility: that such large fields were characteristic of, and emanated from, a pre-main sequence, T-Tauri stage Sun.

The details of our experiments will be published elsewhere. The NRM of lunar rock 72215 was found to be very stable¹¹, and the remanences carried by the desirably high coercivity

Table 1 Palaeointensity values from 72215

Subsample no.	<i>N</i>	<i>H</i> (oersted)	s.d. (02)	Range (oersted)
72215,56	7	0.55	0.10	0.74–0.44
46	7	0.41	0.17	0.60–0.21
93	8	0.28	0.07	0.37–0.18

range of 100–800 oersted were used to calculate palaeointensities. Table 1 lists three palaeointensity values, which are roughly 0.3, 0.4, and 0.5 oersted respectively. Within one standard deviation, the values 0.3 and 0.4 obtained from subsamples 72215,46 and 72215,93 do not differ significantly from each other. The value of roughly 0.5 oersted obtained from 72215,56 is, however, different and may be expected to represent the lunar palaeointensity during the formation of the boulder (4.0×10^9 yr BP); the mean palaeointensity of 0.35 oersted obtained from the other two subsamples may pertain to an older time. We prefer however, to adopt a more conservative attitude: that of calculating an average palaeointensity of 0.4 oersted from all the three subsamples, and assigning them all an age of approximately 4.0×10^9 yr. In the present experiment, for the first time, three subsamples from one rock (72215) have been heated to beyond their respective maximum blocking temperatures, and anhysteretic permanent magnetisation (ARM) curves before and after heating have shown that little change in the magnetic carriers took place during the heating. The deduced palaeointensities are based on comparisons of laboratory TRMs in a known field, with the NRM acquired as TRMs during the assembly of the boulder.

Leaving aside other palaeointensity measurements involving approximate methods such as simulating the TRM of the lunar samples with an ARM (refs 12–14) or induced permanent magnetisation (IRM) (ref. 15), there are four determinations involving complete heatings to the highest blocking temperatures of lunar samples with approximate ages of 4.0×10^9 yr. Our measurements from the three subsamples reported here indicate a value of 0.4 oersted. The other reliable measurement¹⁶, taken from a single piece of 62235,53 (dated at 3.9×10^9 yr) yielded a value of 1.2 oersted. A replotting of those data to meet criticisms¹⁵ does not change the observed value by more than 20%. Thus, we need to find a source for a rather large, ancient lunar magnetic field at $\sim 4 \times 10^9$ yr BP. The magnitude of this field could be as low as 0.4 oersted (our measurements) or as high as 1.2 oersted (Collinson *et al.*¹⁶). In either case, the required fields are approximately 10^4 times greater than present-day interplanetary or solar flare fields.

Sonnett and Runcorn¹⁷ have outlined a rather ingenious mechanism, which could generate a lunar, iron-core dynamo, using inductive heating as a heat source, in a small accreting Moon. It meets one of the difficulties^{15,18} of an operating dynamo in the young lunar core. The present state of knowledge in theoretical magnetohydrodynamics is, however, such that we cannot convincingly say whether such a small dynamo would be able to produce fields on the order of 1 oersted (see ref. 14). In our opinion, however, if we have to choose between a lunar core dynamo and the field of a permanently magnetised, lunar silicate core, once exposed to an even larger primordial field, the first alternative seems more preferable. Strangway and Sharpe⁷ have calculated that a primordial field strength of 10 oersted would have been needed to produce that degree of magnetisation in the silicate core (with 3% by weight of iron) which would lead to an ambient palaeointensity of 0.02 oersted at the lunar surface. Such a low palaeointensity is indicated¹⁹ by an apparently reliable thermal determination on a young (3.3×10^9 yr) lunar breccia, 15498,36. But using the latest value²⁰ of iron content for lunar interior (9% by weight) and our lunar palaeointensity of 0.4 oersted at 4.0×10^9 yr, the updated value of the postulated primordial solar field has to be as large as 75 oersted. Faced with this dilemma of generating 0.4 oersted (or as much as 1.2 oersted) at the lunar surface by invoking either a primordial field of 75 oersted uniform over 10^3 km, or a marginally plausible, central iron-core dynamo that can generate 0.4 oersted at the lunar surface, we prefer the latter model.

A third solution may be even more acceptable than those two. First, we draw attention to the apparent coincidence between the range of 0.4 to 1.2 oersted for a lunar surface field at 4.0×10^9 yr BP, and the reported^{8–10} range of 0.2–1.1 oersted for

palaeointensities determined from carbonaceous chondrites approximately 4.4×10^9 yr old. The latter values are $\sim 10^4$ times greater than the present interplanetary field, and if such a field were attributable to a pre-main sequence, T-Tauri stage Sun (with no sector structure of the solar field), it could indeed be the same large, early Solar System field postulated²¹ to explain the smaller solar angular momentum of that time. Could the lunar surface at 4.0×10^9 yr BP not, in fact, have recorded the same early field? Alternatively, the ambient solar field could have decayed from 1 oersted at 4.4×10^9 yr BP to, say, 0.1 oersted at 4.0×10^9 yr BP. The apparently higher (0.4–1.2 oersted) ambient field at the lunar surface would then be attributable to an amplification of the 0.1-oersted field by 'dusty' plasma dynamos operating on the lunar surface, and generated by the intense collisions^{22,23} which seem to have gone on at the lunar surface till 4.0×10^9 yr BP (ref. 24).

There is one apparently strong objection to this hypothesis. Lunar rocks aged $4.0\text{--}3.2 \times 10^9$ yr old have been found to be magnetised. Thus, it is commonly assumed that the same source of the lunar surface field had to operate continually over the given time range. We do not agree with that contention, and point to a possible recorded drastic decrease in the lunar field after 4.0×10^9 yr BP. The palaeointensity¹⁹ of 0.02 oersted at 3.3×10^9 yr BP is the single reliable, thermally determined value, at present available for those younger times. Future palaeointensity studies may confirm such a low (by two orders of magnitude) value, and thus point to quite a different source for the lunar field, such as the magnetohydrodynamic amplification of a 0.002-oersted ambient solar field, or even the presence of an older underlying crust, magnetised at an earlier time. Regarding the latter, it should be recalled that station magnetometer readings as large as 0.003 oersted produced locally by the magnetised crust were measured by the Apollo-16 astronauts²⁵. So far as the present work is concerned, we believe to have presented credible evidence for a large magnetic field during early lunar history which could most easily come from a pre-main sequence T-Tauri Sun. In as much as we do not have to invoke an external field that was both dipolar and uniform over the Moon as a whole, our conclusion here does not invalidate the suggestion²⁶ that the global lunar magnetic anomaly record could not be attributable to an external, uniform, dipolar field.

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SUBIR K. BANERJEE
JAMES P. MELLEMA

Department of Geology and Geophysics,
University of Minnesota,
Minneapolis, Minnesota 55455

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- 1 Shaw, J., *Geophys. J. R. astr. Soc.*, **39**, 133 (1974).
- 2 Leich, D. A., Kahl, S. K., Kirschbaum, A. R., Niemeyer, S., and Phinney, D., *The Moon* (in the press).
- 3 Compston, W., Foster, J. J., and Gray, C. M., *The Moon* (in the press).
- 4 Nunes, P. D., and Tatsumoto, M., *The Moon* (in the press).
- 5 Runcorn, S. K., *et al.*, *Proc. R. Soc. A*, **325**, 157 (1971).
- 6 Runcorn, S. K., and Urey, H. C., *Science*, **180**, 636 (1973).
- 7 Strangway, D. W., and Sharpe, H. A., *Nature*, **249**, 227 (1974).
- 8 Banerjee, S. K., and Hargraves, R. B., *Earth planet. Sci. Lett.*, **17**, 110 (1972).
- 9 Butler, R. F., *Earth planet. Sci. Lett.*, **17**, 120 (1972).
- 10 Brecher, A., in *On the origin of the Solar System* (edit. by Reeves, H.), 260 (Centre Nationale de la Recherche Scientifique, Paris, 1972).
- 11 Banerjee, S. K., and Swits, G., *The Moon* (in the press).
- 12 Banerjee, S. K., and Mellema, J. P., *Earth planet. Sci. Lett.*, **23**, 177 (1974).
- 13 Banerjee, S. K., and Mellema, J. P., *Earth planet. Sci. Lett.*, **23**, (1974).
- 14 Stephenson, A., and Collinson, D. W., *Earth planet. Sci. Lett.*, **23**, 220 (1974).
- 15 Fuller, M., *Rev. Geophys. Space Phys.*, **12**, 23 (1974).
- 16 Collinson, D. W., Stephenson, A., and Runcorn, S. K., *Proc. 4th Lunar Sci. Conf.*, **3**, 2962 (1973).
- 17 Sonnett, C. P., and Runcorn, S. K., *The Moon*, **8**, 308 (1973).
- 18 Murthy, V. R., and Banerjee, S. K., *The Moon*, **7**, 149 (1973).
- 19 Gose, W., Strangway, S. W., and Pearce, G. W., *The Moon*, **7**, 196 (1973).
- 20 Parkin, C. W., Daily, W. D., and Dyal, P., *Proc. 5th Lunar Sci. Conf.*, **3**, 2761 (1974).
- 21 Fowler, W. A., Greenstein, J. L., and Hoyle, F., *Geophys. J. R. astr. Soc.*, **6**, 148 (1962).
- 22 Hide, R., *The Moon*, **4**, 39 (1972).
- 23 Meadows, A. J., *Nature*, **237**, 274 (1972).
- 24 Hartung, J. B., in *Lunar Science VI*, 337 (The Lunar Science Institute, Houston, 1975).
- 25 Dyal, P., Parkin, C. W., Colburn, D. S., and Schubert, G., *Apollo 16 Preliminary Science Report, NASA Spec. Publ.*, **315**, 11–1 (1972).
- 26 Runcorn, S. K., *Nature*, **253**, 701 (1975).

Radiocarbon evidence for deglaciation in north-western Himalaya, India

SINCE the publication of the first post-glacial pollen diagram from Toshmaidan (74°31'E, 33°56'N, ~ 3,120 m OD) in the Kashmir Valley by one of the authors¹, the age of the pollen sequence has been a bone of contention²⁻⁴. The organic deposits overlie a sequence of bluish-grey buttery clay and crushed rock, and were suggested¹ to date from the last deglaciation. The pollen sequence was divided into eight stages, and showed a cold-warm-cold climatic sequence, which was similar to previous findings on post-glacial climate⁵. Here we present radiocarbon dates on these deposits, and these confirm our previous work.

In early June 1973, an expedition to Kashmir was organised to obtain radiocarbon dates of the inorganic-organic interface, as well as the sedimentary sequence in the mire, at Toshmaidan, and a position stratigraphically close to the original point was selected for collecting samples. The samples dated were subjected to pollen analysis for correlation with the original pollen diagram from the site.

The results of the radiometric dating are shown in Table 1. All but one sample were dated at between ~ 9,500–15,000 b.p., showing that the sedimentation of the organic deposit at Toshmaidan started long before the Neolithic period. The samples which were pollen analysed showed that all samples predating 9,500 b.p. belonged to the time of the first four pollen stages (a–d) (Table 1). The next sample above, however, correlated with the seventh stage (g) of the pollen sequence, and gave a date of $2,790 \pm 160$ b.p. As none of the samples showed any similarities with stages e and f it was suggested that there was an erosional break between samples PRL-2(B) ($2,790 \pm 160$ b.p.) and PRL-3 ($9,650 \pm 245$ b.p.) which correlated with stages g and d respectively. The gap of nearly 7,000 yr between the radiocarbon dates from the above two samples (Table 1) confirmed the presence of the break in sequence. Because of this disconformity in the sequence it is not possible to calculate the rates of sedimentation in the whole sedimentary profile. This also means that pollen stages e and f in Singh's pollen diagram cannot yet be dated accurately. Nevertheless the dates for these stages cannot be outside the range set by PRL-2(B) ($2,790 \pm 160$ b.p.) and PRL-3 ($9,650 \pm 245$ b.p.), and therefore the stages could still represent broadly the mid-postglacial time to which they had been assigned in ref. 1. Similarly stage h, the topmost stage, can now be considered to postdate $2,790 \pm 160$ b.p. Radiocarbon dates from samples comparable with stages a–c fall between ~ 14,000 and 15,000 b.p. and with stage d between 9,500 and 11,000 b.p.

Terminal dates for the pollen stages all remain to be calibrated but it may not be possible to do so accurately without a detailed pollen analysis and fresh radiocarbon dating from the site.

The most significant point to emerge from the present study has been the evidence of deglaciation at Toshmaidan which is shown to have been in progress at ~ 3,120 m OD in Kashmir, ~ 14,000–15,000 yr b.p. This date, from the contact between inorganic and organic sediments, is in agreement with the dates generally associated with deglaciation following the last glacial period in several parts of the world⁶. The regional significance of the date for deglaciation from Toshmaidan will become clear as more dates become available from other parts of the Himalayan region. In the global context, the date of deglaciation from Kashmir broadly falls in line with dates obtained from similar elevations in New Guinea⁷ and Ruwenzori, Uganda⁸ in the tropics. There are, however, no available sites providing information on age of deglaciation situated at similar latitude and altitude as Toshmaidan, and, as a result, exact parallels have not yet been found.

GURDIP SINGH

Research School of Pacific Studies,
Australian National University,
Box 4 P.O. Canberra, ACT, Australia

D. P. AGRAWAL

Physical Research Laboratory,
Ahmedabad, India

Received October 7, 1975; accepted January 16, 1976.

¹ Singh, G., *Palaeobotanist*, 12 (1), 73–108 (1963).

² Vishnu-Mittre, *Palaeobotanist*, 15 (1–2), 157–175 (1966).

³ Vishnu-Mittre, *Geophytology*, 4 (1), 46–53 (1974).

⁴ Vishnu-Mittre, and Sharma, B. D., *Palaeobotanist*, 15 (1–2), 185–212 (1966).

⁵ Post von, L., *New Phytol.*, 45, 193–217 (1946).

⁶ Mercer, J. H., *Quat. Res.*, 2, 15–24 (1972).

⁷ Hope, G. S., and Peterson, J. A., in *Quat. Studies* (edit. by Suggate, R. P., and Cresswell, M. M.), 155–162 (The Royal Society of New Zealand, Wellington, 1975).

⁸ Livingstone, D. A., *Ecological Monographs*, 37, 25–52.

Eastern Greenland basalts and their supposed plume origin

It has been suggested^{1,2} that the eastern Greenland flood basalts had their origin in plume activity centred about a triple junction of which Kangerdlugssuaq (Fig. 1) is the failed arm. The suggestion is reviewed here in light of the petrological characteristics and spatial distribution of the basalts.

The main outcrop of lavas is on the Blossville coast in the 450 km between Kangerdlugssuaq and Scoresby Sund, though scattered, less well known outcrops are found along the 800 km of coast between Kialineq and Shannon Isle. The lavas were extruded during an early stage of the opening of the North Atlantic from sources on the seaward side of the present outcrops. They thin inland from the coast, where estimates³

Table 1 Radiocarbon data from Toshmaidan

PRL number	Serial number	Depth (cm)	Material	Sieve fraction (μm)	Pollen stage (ref. 1)	Radiocarbon date (yr b.p.) (λ taken as 5,568 yr)
2(B)	II	15–35	Peat	< 420	g	2,790 ± 160
3	III	50–70	Peat	< 420	d	9,650 ± 245
4(B)	IV	75–90	Peat	< 420	d	10,005 ± 340
						— 380
5	V	125–140	Peat	< 420	d	11,360 ± 585
						— 600
7	VII	205–220	Fine organic mud	—	c	13,980 ± 520
						— 565
9	IX	280–295	Clay mud	—	c	15,250 ± 760
						— 820
10	X	317–327	Clay mud	—	a–b	14,760 ± 1015
						— 925
12	XII	337–350	Blue-grey lacustrine clay	—	a–b	13,850 ± 900
						— 785

of their thickness in the neighbourhood of Ryberg Fjord indicate a maximum of 9 km. Though Nielsen⁴ has suggested that thickness estimates may be overgenerous because of a failure to take into account faulting, we do not believe that the figures are significantly in error. The base of the pile is submarine and the upper 6 km comprises dominantly, but not exclusively, subaerial flows. Allowing for several kilometres of thinning between Kangerdlugssuaq and Scoresby Sund, and taking into account basalts on the coastal shelf, the present volume along this 450 km of coast is estimated as approximately $6 \times 10^5 \text{ km}^3$.

Palaeontological dating using marine microfossils of the submarine base of the lava pile in the Kangerdlugssuaq–Ryberg Fjord area³ and marine horizons in the top of the pile at Kap Dalton⁵, shows that the effusive activity occurred in latest Palaeocene time over a very restricted time interval, the best estimate of which is approximately 3 Myr. It may be that the activity took place within one geomagnetic reversal³, and if that is so then even 3 Myr may be an overestimate.

Using the best available estimate of 3 Myr, however, the volume of basalts erupted each year in this one area of flood basalts is 0.2 km^3 , well in excess of the estimated maximum discharge of the Icelandic plume model⁶, and as shown in the reconstruction of the North Atlantic (ref. 7, and Fig. 2) the actual extent of the flood basalts must have been much greater. Particularly intense activity is indicated and there is no evidence in the lava pile of regional evolution with time from alkaline to tholeiitic (of the sort found, for example, at the Afar triple junction¹²).

Analyses of subaerial basalts from a typical section in the area of Wiedemann Fjord (Table 1) show a content of large ionic lithophile elements (LILE) characteristic of 'hot spot' basalts, as distinct from relatively LILE-depleted mid-ocean ridge basalts⁸. Published analyses of subaerial basalts from the area of Scoresby Sund⁹, are comparable. Schilling^{10,11} has suggested that 'hot spot' basalts typically have a lanthanum-samarium enrichment factor ($\text{La}/\text{Sm}_{\text{EF}} > 1$) and he has presented analyses from Scoresby Sund which show this feature¹¹. The Wiedemann Fjord analyses likewise show a $\text{La}/\text{Sm}_{\text{EF}} > 1$, and also the light rare earth pattern typical of 'hot spot' basalts (Fig. 3). Schilling and Noe-Nygaard¹¹ have shown that the Lower and Middle Series basalts of the Faeroe Islands also have $\text{La}/\text{Sm}_{\text{EF}} > 1$ and are comparable with the Scoresby Sund basalts, though, as they pointed out, Scoresby Sund in the Atlantic reconstruction is significantly distant from the Faeroes.

Tholeiitic basalts with these characteristics are typically found at oceanic hot spots such as Iceland, or occur late in the process of crustal thinning, as in the Afar region^{12,13}. They show no evidence of continental crustal influence in their chemistry and satisfy the criteria of volume and compositional homogeneity indicative of basalts generated directly by mantle fusion. The Greenland basalts overlie continental crust but their chemistry and the low pressure regime necessary for their voluminous generation by direct mantle melting^{14,15} indicate that they were erupted from the embryonic ocean forming offshore from the present outcrop after thinning of the continental crust. The evidence is that this part of Greenland never attained any significant elevation above sea level before or during the effusive activity^{3,5}, and it is not difficult to envisage the lavas spreading on to the continental crust and thinning away from their source area.

The original plume hypothesis of Morgan¹⁶ postulated plumes of the order of 150 km diameter rising from the deep mantle. As developed by Schilling¹⁰ and Vogt¹⁷ for Iceland, a much larger plume of geochemically undepleted mantle, 200 km in radius, rises beneath Iceland and provides material which spreads preferentially away beneath the mid-ocean ridge. Geochemical anomalies of decreasing magnitude are found up to 400 km away from Iceland¹⁰ (that is, up to three times the plume radius from its centre), beyond which normal (relatively depleted) mid-ocean ridge lavas become dominant. Schilling¹⁰

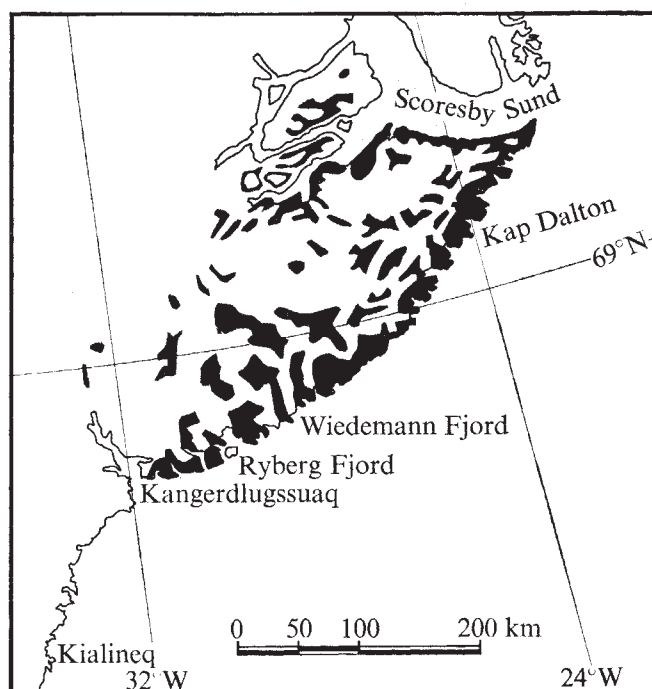


Fig. 1 Localities and basalt distribution between Kangerdlugssuaq and Scoresby Sund.

attributed the systematic variation in rare earth and minor element concentrations, which he documented along the Reykjanes Ridge axis, to the mixing of relatively undepleted (plume) and depleted (asthenosphere) materials or their partial melt products (though later developments^{18,19} have shown that the variations may be step-like rather than gradational). Flow rates away from the plume beneath the mid-ocean ridge have

Fig. 2 Bott and Watts⁷ reconstruction of the North Atlantic, showing position of Faeroe Isles and probable distribution of basalts 55 Myr BP.

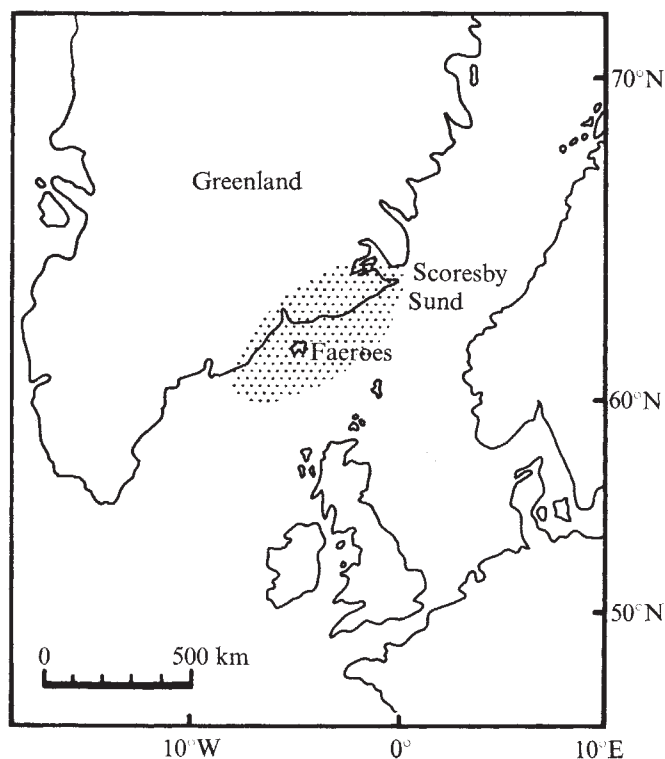


Table 1 Analyses of subaerial basalts from a typical section near Wiedemann Fjord

	Wiedemann Fjord*	Scoresby Sund†
SiO ₂	47.51 ± 0.39	49.0
Al ₂ O ₃	13.74 ± 0.34	13.8
TiO ₂	2.21 ± 0.23	1.9
Fe ₂ O ₃	4.35 ± 0.65	6.2
FeO	8.84 ± 0.85	8.2
MgO	7.07 ± 0.38	6.6
CaO	11.37 ± 0.72	11.1
Na ₂ O	2.26 ± 0.31	2.3
K ₂ O	0.29 ± 0.19	0.3
MnO	0.17 ± 0.06	0.2
P ₂ O ₅	0.21 ± 0.02	0.2
La/Sm _{EF} *	1.38	0.15

*Mean ± s.d. of 11 basalts from Wiedemann Fjord (analysts V. A. Somogyi and R. Kanaris-Sotirion).

*La/Sm_{EF} for 10 of these specimens (analyst J. E. Whitley).

†Average of 37 analyses from Scoresby Sund.

been estimated¹⁷ at 10–20 cm yr⁻¹, and 5–7 Myr must have elapsed for the geochemical anomalies to reach the furthest distance from the plume at which they can be detected. If the plume model is applied to Greenland 55 Myr BP, then the distribution of the basalts would likewise indicate preferential movement of the plume material along the developing lithospheric weakness from which the lavas erupted. If a plume of the large 200-km radius and flow characteristics suggested for Iceland was centred about Kangerdlugssuaq, then several million years would be expected to elapse between the initiation of magmatic activity over the plume and the build-up of the voluminous extrusions actually found along the length of the coast. The evidence, however, suggests that chemically similar, undepleted magmas erupted in large volumes simultaneously between Kangerdlugssuaq and Scoresby Sund, the whole effusive activity occupying approximately a mere 3 Myr. On the plume hypothesis, an escalation in plume intensity or an unlikely increase in dimensions to something double that for Iceland would be necessary.

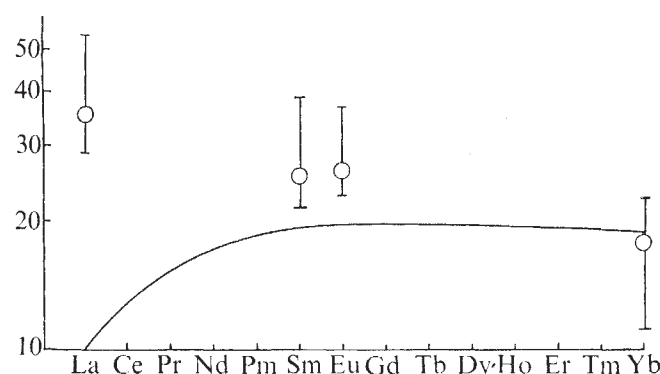


Fig. 3 Pattern of chondrite normalised, selected rare earths for Wiedemann Fjord basalts, showing average and spread (analyst J. E. Whitley). Lower line, chondrite normalised, mid-ocean ridge average pattern²².

It is concluded that in their spatial and temporal characteristics the eastern Greenland flood basalts do not easily conform to the model of a narrow plume beneath continental crust. The rise of a discrete mantle blob, as opposed to a long lived plume (see ref. 11), a model which offers an explanation of sudden chemical changes with time, has similar difficulties of dimensions and timing when applied to the eastern Greenland–Faeroes situation. A convective system of wide lateral extent, or perhaps the linking of several lithothermal systems into a major convection system^{20,21} seems preferable. Nevertheless the problem of separate magma sources for hot spot and mid-ocean ridge

basalts, or the alternative of appealing to phlogopite stability and disequilibrium melting¹⁵, still remains.

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P. E. BROWN

Department of Geology and Mineralogy,
University of Aberdeen,
Aberdeen AB9 1AS, UK

J. E. WHITLEY

Scottish Universities Research and Reactor Centre,
East Kilbride, Glasgow, UK

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- Brooks, C. K., *Nature phys. Sci.*, **244**, 23 (1973).
- Burke, K., and Dewey, J. F., *J. Geol.*, **81**, 406–433 (1973).
- Soper, N. J., Higgins, A. C., Downie, C., Mathews, D. W., and Brown, P. E., *J. geol. Soc. Lond.*, **132**, 85–104 (1976).
- Nielsen, T. F. D., *Nature*, **253**, 182–184 (1975).
- Soper, N. J., and Coasta, L. L., *Grønland geol. Unders. Rapp.* (in the press).
- Vogt, P. R., *Nature*, **240**, 338–342 (1972).
- Bott, M. H. P., and Watts, A. B., *Rep. No. 70/14, Inst. geol. Sci.* (1971).
- Brooks, C. K., and Jakobsson, S. P., in *Geodynamics of Iceland and the North Atlantic Area* (edit. by Kristjánsson, W. W.), 139–154 (Dordrecht-Holland, Amsterdam, 1974).
- Fawcett, J. J., Rucklidge, J. C., and Brooks, C. K., *Meddr Grønland*, **195**(6), 1–54 (1973).
- Schilling, J. G., *Nature*, **242**, 566–571 (1973).
- Schilling, J. G., and Noe-Nygaard, A., *Earth planet. Sci. Lett.*, **24**, 1–14 (1974).
- Gass, I. G., *Phil. Trans. R. Soc.*, **267**, 369–381 (1970).
- Schilling, J. G., *Nature phys. Sci.*, **242**, 2–5 (1973).
- Green, D. H., *Phil. Trans. R. Soc.*, **268**, 707–725 (1971).
- Flower, M. F. J., Schmincke, H. U., and Thompson, R. N., *Nature*, **254**, 404–406 (1975).
- Morgan, W. J., *Bull. Am. Petrol. Geol.*, **56**, 203 (1972).
- Vogt, P. R., in *Geodynamics of Iceland and the North Atlantic* (edit. by Kristjánsson, W. W.), 105–126 (Dordrecht-Holland, Amsterdam, 1974).
- Hart, S. R., and Schilling, J. G., *Yb. Carnegie Instn. Wash.*, **72**, 259–262 (1973).
- Hart, S. R., Schilling, J. G., and Powell, J. L., *Nature phys. Sci.*, **246**, 104–107 (1973).
- Gass, I. G., *Phil. Trans. R. Soc.*, **271**, 131–140 (1972).
- Bott, M. H. P., in *Implications of Continental Drift to the Earth Sciences* (edit. by Tarling, D. H., and Runcorn, S. K.), 175–189 (Academic, London, 1973).
- Schilling, J. G., *Phil. Trans. R. Soc.*, **A268**, 663–706 (1971).

Late-glacial and postglacial sedimentation in the Peris-Padarn Rock Basin, North Wales

BOREHOLES sunk in the Llyn Peris Trough, North Wales, have revealed considerable thicknesses of Quaternary deposits, including glacial lake rhythmites, sands and gravels, together with substantial amounts of organic lake mud and wood debris peats rich in pollen and macrofossils. Preliminary sedimentological and palaeontological analysis and radiometric dating indicates that the sedimentological record extends from the period of ice wastage in the valley heads into the Christian era.

The gravels, sands, silts and peats were deposited in a rock basin produced by glacial overdeepening of the Cambrian shales, slates and gritstones. The lowest lithostratigraphical unit consists of laminated lacustrine clay-silts which are locally sandy and gravelly. They are glacialacustrine rhythmites predominantly of diatatic type and, so far, they have proved to be barren of pollen and macroscopic organic remains. They exceed a thickness of 27 m (borehole EP7: Fig. 1) and they rest on rock-head at about 35 m OD in the vicinity of borehole EP4. In thin section, both coarse and fine laminae can be seen to contain an appreciable amount of clay-sized material, the range of grain size in the coarser layers (1–10 μm) being about one order of magnitude larger than in the finer laminae. Grain counts of the relatively few clasts with long axes greater than 0.2 mm revealed a predominance of feldspars, muscovite, quartz with some grains of magnetite, chlorite and partly altered biotite. The finer material in the coarser layers and the material in the finer layers was found to be dominantly of quartz with feldspars, muscovite and some chlorite. The finer laminae include a notable amount of very fine grained clay mineral. Analysis of this clay mineral component by X-ray diffraction showed a dominance of muscovite, chlorite and kaolinite, in addition to

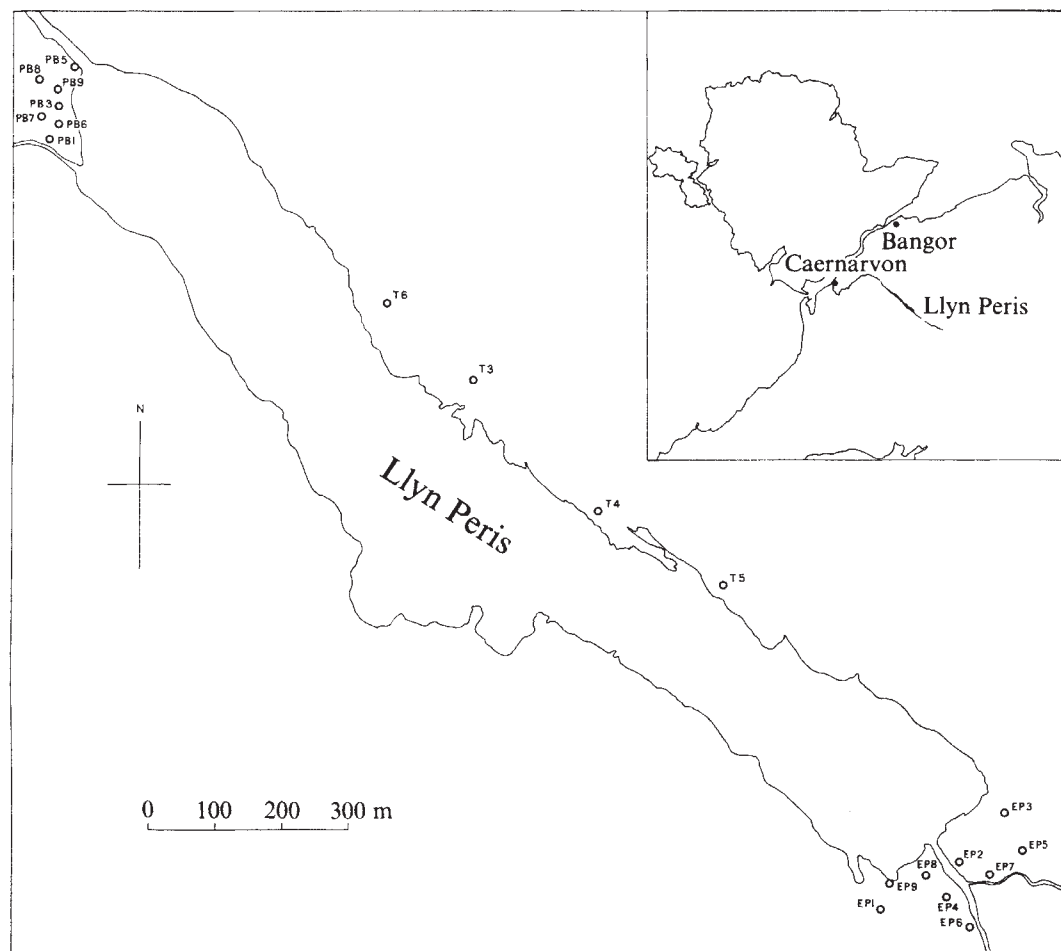


Fig. 1 Location of Llyn Peris and sites of boreholes.

quartz. The kaolinite has a high crystallinity index, indicating a clastic origin, attributable to the grinding action of ice. There is little amorphous clay and little difference in the clay mineralogy of the coarse and finer layers. The microfabric of the silts and clays is being investigated using the scanning electron microscope.

Although severely disturbed in local areas because of overburden from slate quarries, the rhythmites make up a regular series of plane beds with only occasional discontinuities caused by local penecontemporaneous erosion and minor faulting.

Varve counting methods have been applied to the total length of the rhythmites in borehole T3. The mean thickness of rhythmite sets (which are assumed to represent deposition over 1 yr, and thus to be true varves) is 12 mm (range 2–50 mm), the number of coarse and fine couplets within each varve varying from 1 to 8. Given that the rhythmite sets are true varves, the results suggest that accumulation extended over a period of 738 yr, or 850 yr if an estimate is included for gaps in the core.

At the head of Llyn Peris and in the large fan at Pont-y-Bala separating Llyn Peris from Llyn Padarn, the rhythmites are overlain by beds of glacial origin. These sediments range in size from very coarse cobble and pebble gravels to thin layers of fine and medium sand associated with the rhythmites and the overlying organic deposits. The constitution of the gravels is dominated by the local bedrock types, the slates and shales giving high average flatness values in all grades, and moderate to high roundness values. Clasts of medium gravel grade and larger show faceted forms. In the case of the grits and vein quartz that is probably attributable to glacial grinding. The medium sand grade, probably the most susceptible to edge

rounding, is moderately to well rounded. The greatest thicknesses of sand and gravel are found in the large fan at Pont-y-Bala, which comprises interlayered sands, gravels, rhythmites and peats.

The sands and gravels are the products of periodically high discharges of meltwater streams which issued from the Peris Glacier above the lakehead and from waning cirque glaciers above Pont-y-Bala¹. This gravel fan is of particular interest. As it is interbedded with rhythmites, it evidently began to accumulate well before the final vestiges of ice disappeared from the adjacent cirques and higher valley heads. The interbedding of the sand and gravels with peat indicates that the fan continued to grow well into the postglacial period. Thus, fan growth imposed a progressively rising water level on the Llyn Peris Basin.

Complete bores and bulk samples of the organic layers (earlier obtained by shell and auger rig) have been examined from sites at both ends of Llyn Peris. Macroscopic examination has revealed three distinct suites of organic deposits.

The Pont-y-Bala suite consists of discontinuous wood peat lenses, between 0.5 m and 2 m thick, interbedded with gravels. Borehole PB6 traversed three such lenses (Fig. 2). The wood, which is of *Betula/Alnus* type, is highly compressed and distorted. This feature, together with the high mineral content of the peat, suggests that the material has been rafted into the Pont-y-Bala fan from adjacent slopes. The East Peris suite from the head of the basin is much more impressive. Borings have revealed organic deposits up to 20 m thick buried by 10–12 m of sands and gravels. Material from the EP7 borehole at depths between 10 m and 30 m (Fig. 3) is highly variable, comprising organic lake muds, wood and debris peat with well preserved

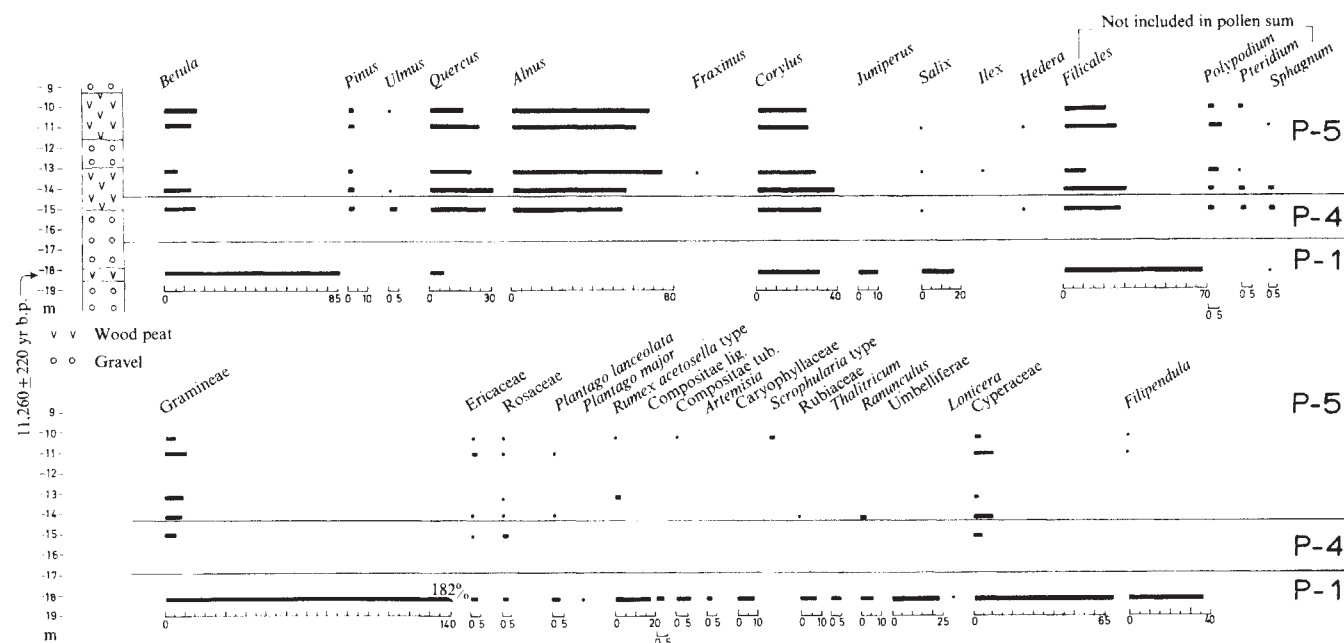


Fig. 2 Tree and non-tree pollen expressed as percentages of total tree pollen for PB6 at depths of 9–19 m.

leaves, and sand and gravel partings. These deposits indicate accumulation *in situ* in a marginal lacustrine environment with a rapidly rising water table. The Central Peris suite of organic deposits is not discussed in detail here. This suite lies beneath Llyn Peris and beneath the Dinorwic slate tip, which has extended out into what was formerly the lake. They comprise fine-grained, dark-grey organic lake muds and silts, typical of a deep lacustrine environment.

Figures 2 and 3 show the pollen diagrams from boreholes PB6 and EP7. The sampling interval was determined by the availability of bulk samples from those cores. Six pollen assemblage zones, Peris 1–6, have been recognised, and their characteristics are summarised in Table 1. They are listed in what is assumed to be chronological order,

although the sequence in PB6 is interrupted because of the discontinuous nature of the deposits.

The earliest assemblage, Peris 1 has a late Weichselian date of $11,260 \pm 200$ yr b.p. (Table 1, B-555), and has similarities with the late-glacial floras described from Cwm Dwythwch and Nant Ffrancon[†].

The assemblage suggests a fairly open environment, as the proportions of pollen of light-demanding plants are high. It is likely that scrub of birch and willow was fairly widespread in the basin. If the theory of rafting is correct some of this material may have originated in the Cwm Dwythwch area, as this lies immediately above the Pont-y-Bala fan. Figure 3 shows the full sequence of pollen assemblage zones (Peris 2 to Peris 6), and these exhibit the standard changes which are associated with the develop-

Table 1 Characteristics of Peris pollen assemblage zones

Pollen assemblage zone	Representative samples	Characteristics	Affinities and dating†
Peris 1 <i>Betula</i> / <i>Filipendula</i> / <i>Rumex</i> assemblage	PB6 18.55 m	<i>Betula</i> > 80% AP.* <i>Filipendula</i> > 30% AP. <i>Rumex</i> > 10% AP Gramineae > 150% AP. AP* values relatively low. Wide variety herb pollens.	Late Weichselian $11,260 \pm 220$ yr b.p.
Peris 2 <i>Betula</i> / <i>Pinus</i> / <i>Corylus</i> assemblage	EP7 29.5 m	<i>Betula</i> > 50% AP. <i>Pinus</i> > 30% AP. <i>Corylus</i> > 100% AP. Herbaceous types well represented but reduced in variety compared with Peris 1.	Flandrian chronozone F I $9,400 \pm 200$ yr b.p. (start zone)
Peris 3 <i>Pinus</i> / <i>Ulmus</i> assemblage	EP7 28.0–28.95 m	<i>Pinus</i> > 40% AP. <i>Ulmus</i> > 10% AP. <i>Betula</i> < 30% AP. <i>Corylus</i> < 60% AP. <i>Alnus</i> rising. Low representation of herbaceous types.	Flandrian chronozone F II
Peris 4 <i>Alnus</i> / <i>Quercus</i> / <i>Ulmus</i> assemblage	PB6 15.0 m EP7 23.75–28.0 m	<i>Alnus</i> > 60% AP. <i>Quercus</i> > 20% AP. <i>Ulmus</i> > 5% AP. Low representation of herbaceous types.	Flandrian chronozone F II
Peris 5 <i>Alnus</i> / <i>Quercus</i> assemblage	PB6 9.4–14.5 m EP7 15.45–22.8 m	<i>Alnus</i> > 60% AP. <i>Quercus</i> > 25% AP. <i>Ulmus</i> < 2% AP. Low representation of herbaceous types. Representation of <i>Betula</i> increases at expense <i>Quercus</i> at top of zone.	Flandrian chronozone F III
Peris 6 Gramineae/ <i>Plantago lanceolata</i> assemblage	EP7 13.72–10.6 m	Gramineae > 75% AP. <i>Plantago lanceolata</i> > 5% AP. A wide variety of herb pollen types represented.	Flandrian chronozone F III $1,750 \pm 100$ yr b.p. (end zone)

* AP, Arboreal pollen.

† Radiocarbon dates provided by Department of Geology, University of Birmingham.

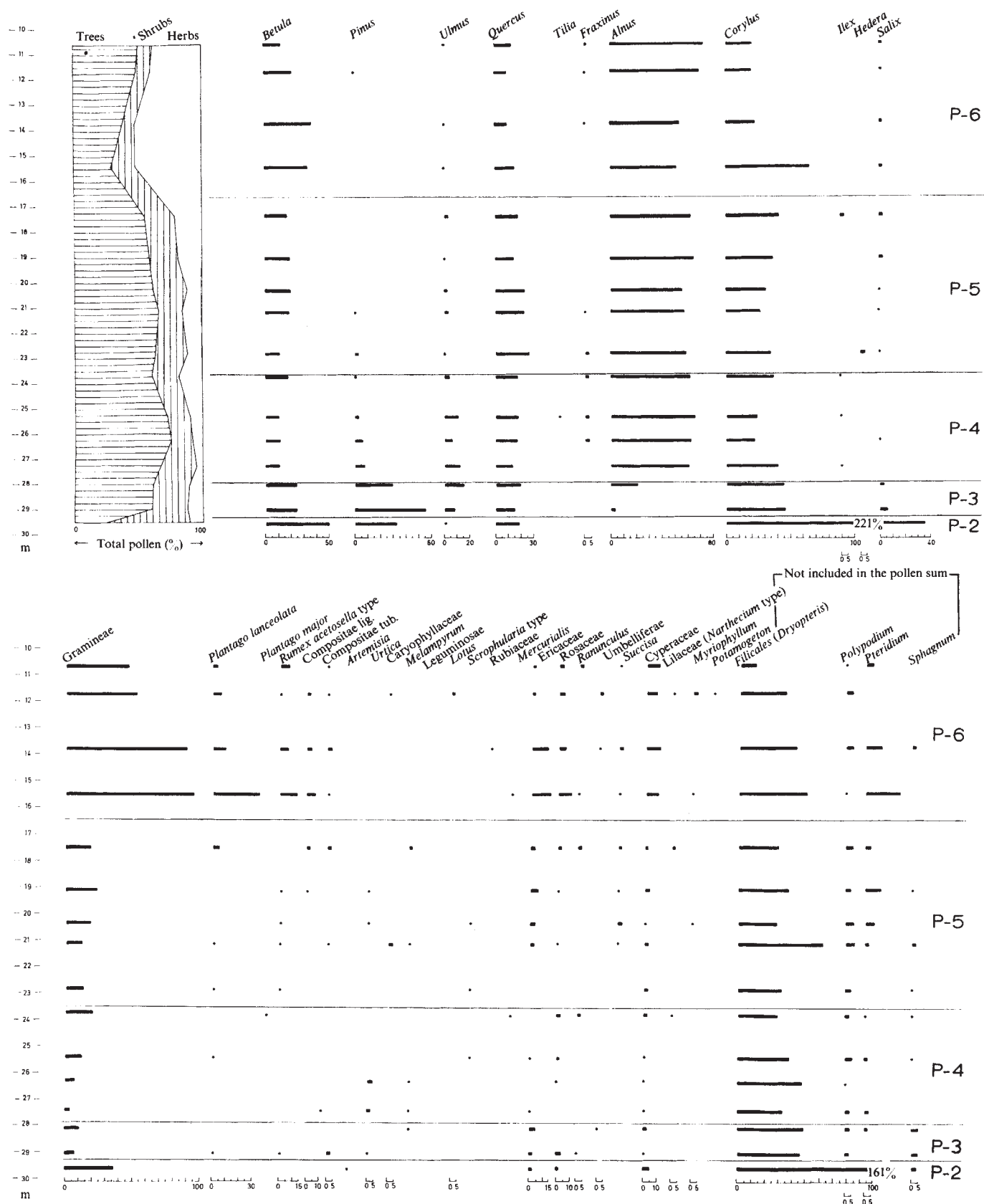


Fig. 3 Tree and non-tree pollen expressed as percentages of total tree pollen for EP7 at depths of 10–30 m.

ment of Flandrian vegetation in the British Isles. Material from Peris 2 has a radiocarbon date of $9,400 \pm 200$ b.p. (Table 1, B-554). The high percentages of *Corylus* pollen typical of this assemblage occur at many sites in the British Isles in Flandrian chronozone Ib (ref. 2). The woodland canopy in the basin was becoming closed at this stage,

although some open areas clearly persisted. Stands of pine existed, with birch and hazel scrub being widespread. Peris 3 is characterised by an increase in *Pinus* pollen at the expense of *Betula*. Pine must have come to dominate the woodlands of the basin in this zone, but oak and elm were spreading and alder was already present in the area.

Mixed oak forest became well established in the Peris-Padarn basin during Peris 4. The expansion of *Alnus* which marks the opening of this zone, is usually assumed to correlate with the FI/FII chronozone boundary around 7,000 yr b.p. The stratigraphical evidence suggests that dense alder carr grew at the eastern end of Llyn Peris at that time, probably extending all around the lake and partly up the slopes as the assemblage is also represented in the rafted material from Pont-y-Bala. The canopy seems to have been very dense with few herbaceous plants surviving. The *Ulmus* decline horizon, assumed to date from around 5,000 yr b.p. (ref. 3) has been used to separate the Peris 4 and Peris 5 assemblage zones. Apart from the decline in elm in the area the forest composition does not seem to have changed markedly. In contrast, the high proportions of pollen of Gramineae and herbs in Peris 6 are indicative of man's interference with the vegetation on a large scale. *Plantago lanceolata* is a herb particularly associated with pastoral farming activities, and it seems probable that the inception of this zone dates from the Bronze Age. Organic material from the top of the zone has a radiocarbon date of $1,750 \pm 100$ yr b.p. (200 AD: B-556).

The sequence of vegetation history follows the pattern already established for western Britain. Further detailed analyses of these and other cores from the area should allow the reconstruction of a complete picture of the ecological development of the basin. Particular attention will be paid to establishing the nature and scale of man's activities in this highland area.

Two features of the deposits of this basin are of outstanding interest. The first is the very great depth of peat, of the order of 20 m, which has accumulated in 9,500 yr at the East Peris site. This can only be accounted for in terms of a very rapidly rising water table which must have been governed by the growth of the alluvial fan at Pont-y-Bala. The second feature of particular interest is the 10 m of gravel which buries these East Peris deposits—gravel which has accumulated in the basin since 200 AD. Certainly, catastrophic events must have been involved in its deposition. It seems that disruption of the vegetation cover on the steep hillsides triggered this wave of sedimentation. Whether this was the product of climatic oscillation or early man is still to be determined.

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HEATHER M. TINSLEY
EDWARD DERBYSHIRE

Department of Geography,
University of Keele,
Keele, Staffordshire ST5 5BG, UK

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¹ Seddon, B., *Phil. Trans. R. Soc.*, B244, 459–481 (1962).

² West, R. G., *New Phytol.*, 69, 1179–83 (1970).

³ Godwin, H., *Phil. Trans. R. Soc.*, A269, 57–75 (1970).

Relationships of Middle and Upper Pleistocene hominids from sub-Saharan Africa

It has long been common to refer to the Broken Hill (Kabwe) and other later Pleistocene African hominids as representative of a "Neanderthaloid" population in the sub-Saharan. In the first account of the Rhodesian skull, Smith Woodward¹ notes similarity to the "Neanderthal or Mousterian race", and this theme has been repeated, with qualifications concerning the extent to which African Neanderthals actually resemble their European relatives^{2–4}.

Brose and Wolpoff⁵ lump all African and Eurasian specimens under the heading "Neanderthal". Following Mourant⁶, a few recent studies^{7,8} have cast doubt on the interpretation; these are based on measurement and use distance statistics which show separation between African and European materials but provide little information about important anatomical differences involved. I report here morphological evidence of the distinctiveness of Broken Hill and its relationships to other African remains. Examination of original fossils from Hopefield (Elandsfontein), Florisbad and the Omo as well as Broken Hill reveals a pattern quite unlike that of Neanderthal crania from Europe.

The Rhodesian skull roughly resembles Neanderthals and other archaic *Homo sapiens*, in that the facial skeleton is large below the massively developed supraorbital torus, the braincase low, and the occiput rather more sharply angulated than in modern man. Broken Hill, however, is extreme in supraorbital development, and the torus is absolutely thicker than in comparable later Pleistocene material (Table 1). Frontal flattening is marked, in spite of some keeling in the midline. The frontal is also relatively narrow as noted by Mourant⁶, so that torus exaggeration produces lateral flare of the anterior temporal lines, and the temporal fossae are deep. These characteristics are not matched in European specimens, and Neanderthals generally have a supratatorial sulcus (ophryonic groove) backed by a prominent, more bulbous frontal region. "Classic" Neanderthal crania also have extensive flattening of the parietal vault near bregma, while there is lateral swelling of the braincase towards asterion. With some variation, there is rearward extension of the occiput to form a bun-like structure. Although the Rhodesian cranium has limited parietal flattening along the posterior course of the sagittal suture, characteristic asterionic bulging of the vault is not apparent, and this part of the braincase seems poorly filled by comparison. Greatest breadth is in the supramastoid region rather than higher on the parietals as in most Neanderthal specimens. Viewed from the side, the occiput is nearly vertical above a strongly developed torus, so that actual bunning of the bone is slight. This has been over-emphasised in discussions in which Broken Hill is labelled an African Neanderthal.

Striking aspects of the Rhodesian face are its biorbital width and extreme length, especially below the nasal opening. But this face has been shown to be less projecting at nasion and subspinale than various Neanderthals measured⁹. Inspection reveals relative flatness of the maxillary frontal processes and the walls below the orbit, whereas in European crania there is obvious retraction of the zygomatic arch relative to the facial midline. Broken Hill has some alveolar prognathism, but only here does protrusion begin to approach that recorded for Neanderthal remains.

There are such departures from "classic" Neanderthal morphology in other African skeletons, though there has been little effort to document them systematically. Parallels between the Rhodesian and Hopefield fossils have been emphasised^{2,10} and these are most obvious in the frontal region. Torus form is certainly similar. The Hopefield parietals are slightly more rounded laterally, but maximum breadth is still low, near the mastoids (which are missing). The base is also broken away, but the form of the occiput seems to be like that of Broken Hill, even if a torus is not so well developed. A fossil with similar morphology is Eyasi I, though this represents a smaller individual, perhaps a female. A cast in the British Museum (Natural History) shows the supraorbital torus to be less robust than in the southern skulls, but in overall appearance Eyasi I is strikingly like Hopefield.

The Omo skeletons¹¹ now provide more information from East Africa, and Omo II has a few traits not seen in Broken Hill. The Omo II frontal bone is substantially broader than

Table 1 Measurements of Middle and Upper Pleistocene hominid crania from East and South Africa

Measurement (mm)	Broken Hill	Hopefield	Omo II	Omo I	Florisbad*	Olduvai Hominid 9
Cranial length	205	200‡				206
Torus thickness†						
central	23	21			15	19
lateral	16	15			12	14
Basion-nasion	108					119‡
Basibregmatic height	127					110‡
Max. cranial breadth	145		147			150
Biauricular breadth	140§		132			135
Max. frontal breadth	118	114	121		132§	
Min. frontal breadth	99	102	108		116§	88
Biorbital chord	125				124§	
Malar height	29				24	
Frontal chord	120	116‡			120	
Frontal angle (degrees)	18	20‡			23	
Parietal chord	111	109	119			
Occipital chord	87		106	101		
Occipital angle (degrees)	40‡		34	35		

All measurements were taken on original specimens. Techniques and definitions are discussed ref. 24.

*The Dreyer reconstruction of Florisbad has been disassembled, and the measurements listed are those which can be obtained from the separate frontal and facial parts. Until a new reconstruction is attempted, dimensions of the orbit and nasal opening cannot be reliably reported.

†Supraorbital torus development is difficult to quantify, and my measurements do not correspond exactly with others reported (for example, ref. 2). One reading is taken in the centre of the orbital margin, though this is not always the point of maximum thickness. The second measurement is taken close to the frontomalar suture, to gauge the extent of lateral attenuation of the torus.

‡These measurements make use of reconstructed landmarks.

§Values obtained by doubling a reading to the midline.

the Rhodesian specimen and quite flat, lacking a supratotal sulcus. The supraorbital margin is intact for only about 4 cm from the left frontomalar suture, and here torus development is slight; however, thickening of the bone towards the centre of the orbit suggests more massive construction in the middle of the face. Outcurving of the anterior temporal lines is not marked, and the temporal fossae are not deep as in Broken Hill and Hopefield. But the skulls are similar in side view, and the vertex lies well forward near bregma. Behind this point, the Omo profile drops evenly away for about half the parietal arc length and then dips again, though the resulting step-like contour is not as obvious as in Broken Hill. There is slight keeling of the parietals in the midline, and there is none of the lambdoid flattening characteristic of European Neanderthals. Instead the parietal wall falls sharply from the temporal line to the mastoid, so that the sides of the cranium are almost concave above the massive mastoids and supra-mastoid crests, and there is no bulging of the parietals at asterion. Occipital curvature is comparable with that of Broken Hill and Hopefield, but the facial skeleton is missing.

Omo I is less complete but has been termed more modern in appearance. Certainly the back of the occiput is higher relative to the reduced nuchal plane, and the entire bone seems less flexed than that of Omo II. Prominent but localised raised areas of muscle insertion on the nuchal surface are misleading, however, and absolute differences in curvature are small (Table 1). The vault is higher and has a more rounded parietal profile from behind. Several frontal fragments cannot be fitted to the parietals but suggest a flattened forehead not unlike that of Omo II.

Given these features of the Omo skulls, several hypotheses regarding their affinities deserve examination. Leakey¹² views the Omo material as separate from "Neanderthaloids" such as Broken Hill and Hopefield. This may be reasonable if only the frontal is considered, but obvious similarities in total pattern between Omo II and the southern remains cannot be ignored. Other workers see a major division within the Omo assemblage and prefer to set only Omo I apart, as more modern than Omo II as well as Broken Hill. Statistical results^{8,13} which seem to support this are based on few measurements or on reconstructions, however, and it is questionable whether a firm case for two lineages in East Africa can be made on the evidence avail-

able. An alternative suggestion⁸ that Omo I is sampled from a more recent segment of the lineage also containing Omo II cannot be verified geologically¹⁴.

While there is uncertainty about Omo I, the remaining sub-Saharan crania including Omo II are not only long and low in outline but distinctive in more specific ways. Frontal flatness (whether or not associated with extreme supra-orbital development), an anteriorly placed vertex coupled with a step-like sagittal contour, near vertical parietal walls above a massive and broad cranial base, and an angulated occiput are all features of a braincase poorly filled by modern standards. The rear of the skull, which lacks asterionic expansion and shows no real occipital bun, is not like that of European Neanderthals, nor is the Broken Hill face, which is broad and devoid of projection in its upper parts. Surely these African hominids are better referred to as archaic than as Neanderthal-like¹⁵, and this term also describes the mandibles usually assigned to the later Pleistocene¹⁶. A formal subspecific designation as *Homo sapiens rhodesiensis* now seems justified on morphological as well as geographic grounds. Recent revisions of dating support this view and suggest that the African material is substantially earlier than the Würm age appropriate for European specimens. Klein¹⁷ argues for a Middle or earliest Upper Pleistocene date at Broken Hill, and the bulk of the Hopefield fauna is also likely to be Middle Pleistocene¹⁸. An aspartic acid racemisation age for a femoral fragment in the Broken Hill assemblage is reported as 110,000 yr (ref. 19), and there is a Th/U date of 130,000 yr for member I of the Kibish Formation in which the Omo skulls were found²⁰.

If a later Middle Pleistocene age can be accepted for this material, comparisons with other earlier African fossils are more convincing. Leakey²¹ notes resemblances of Broken Hill and Hopefield to hominid 9 from bed II at Olduvai, but this important find has never been described fully. Hominid 9 is about equal in length to the Rhodesian skull and exceeds it in maximum breadth across the base by 5 mm. It is also lower and more rugged in general morphology, and inion lies well above the horizontal plane. The occiput is more strongly curved, and the extensive nuchal surface merges evenly on to the broad posterior faces of the pyramidal mastoid processes. That this heavy flattened cranium is easily distinguished from Broken Hill is not surprising, if the Olduvai hominid is roughly 0.9–1.0 Myr in age²². But there are points of definite similarity, particu-

larly in the frontal region. Torus development and post-orbital constriction are marked on hominid 9, though the straight brow is not quite so thick as that of Rhodesian man. There is also general resemblance in occipital proportions to the later skulls, especially Omo II, although this braincase is more rounded at the back and bears modern looking mastoids. Since the functional significance of such craniofacial likeness is uncertain, the issue of relatedness must be approached with caution. But, the still archaic skulls of *Homo sapiens rhodesiensis* can be regarded as somewhat expanded, higher versions of the Olduvai vault, and the populations sampled at Broken Hill and in the Omo are probably evolved from local groups of early Mid-Pleistocene *Homo erectus*.

Where some individuals (for example, Omo I) depart from the archaic morphology of Broken Hill, there is a suggestion of continuity with more recent hominids. The Florisbad frontal exceeds even that of Omo II in breadth, and brow ridges must be more comparable in size with the (incomplete) Omo structures than with those of Rhodesian man. Glabellar protrusion is similar to Omo I. The Florisbad forehead is, however, not as evenly flattened anteriorly, and in torus form and temporal fossa depth this specimen rather recalls the architecture of Broken Hill and Hopefield. Even if Florisbad is correctly dated at 39,000 yr (ref. 23), it is far from fully modern in construction. Facial fragments should provide more information, and fresh study will tie Florisbad more firmly into the succession of later Pleistocene African populations.

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G. P. RIGHTMIRE*

Department of Anthropology,
State University of New York,
Binghamton, New York 13901

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*Present address: Department of Archaeology, University of Cape Town, Private Bag, Rondebosch, Cape Province, South Africa.

- ¹ Woodward, A. S., *Nature*, **108**, 371–372 (1921).
- ² Singer, R., in *Hundert Jahre Neanderthaler* (edit. by von Koenigswald, G. H. R.), 52–62 (Kemink en Zoon, Utrecht, 1958).
- ³ Tobias, P. V., in *Evolution and Hominisation* (edit. by Kurth, G.), 176–194 (Fischer, Stuttgart, 1968).
- ⁴ Mann, A., and Trinkaus, E., *Yb. Phys. Anthropol.*, **17**, 169–193 (1973).
- ⁵ Brose, D. S., and Wolpoff, M. H., *Am. Anthropol.*, **73**, 1156–1194 (1971).
- ⁶ Mourant, G. M., *Ann. Eugen.*, **3**, 337–360 (1928).
- ⁷ Bilsborough, A., *J. hum. Evol.*, **2**, 387–403 (1973).
- ⁸ Stringer, C. B., *J. archaeol. Sci.*, **1**, 317–342 (1974).
- ⁹ Howells, W. W., *Ninth International Congress of anthropological and ethnological Sciences* (in the press).
- ¹⁰ Wells, L. H., in *Proc. 3rd Pan-African Cong. Prehistory* (edit. by Clark, J. D.), 172–174 (Chatto and Windus, London, 1957).
- ¹¹ Day, M. H., *Nature*, **222**, 1135–1138 (1969).
- ¹² Leakey, L. S. B., in *The Origin of Homo sapiens* (edit. by Bordes, F.), 25–29 (UNESCO, Paris, 1972).
- ¹³ Day, M. H., *Acad. Naz. L.*, **182**, 87–95 (1973).
- ¹⁴ Butzer, K. W., *Nature*, **222**, 1133–1135 (1969).
- ¹⁵ Howells, W. W., *Am. Anthropol.*, **76**, 24–38 (1974).
- ¹⁶ Tobias, P. V., *Am. J. phys. Anthropol.*, **34**, 335–368 (1971).
- ¹⁷ Klein, R. G., *Nature*, **244**, 311–312 (1973).
- ¹⁸ Butzer, K. W., *S. Afric. J. Sci.*, **69**, 234–238 (1973).
- ¹⁹ Bada, J. L., Schroeder, R. A., Protsch, R., and Berger, R., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 914–917 (1974).
- ²⁰ Butzer, K. W., Brown, F. H., and Thurber, D. L., *Quaternaria*, **11**, 15–29 (1969).
- ²¹ Leakey, L. S. B., *Nature*, **189**, 649–650 (1961).
- ²² Leakey, M. D., *Olduvai Gorge III. Excavations in Beds I and II*, 1960–1963 (Cambridge University Press, Cambridge, 1971).
- ²³ Protsch, R., thesis, Univ. California, Los Angeles (1973).
- ²⁴ Rightmire, G. P., *Am. J. phys. Anthropol.*, **42**, 351–369 (1975).

A spatial approach to insect population dynamics

THERE are two aspects of the regulation of animal numbers: first, how the numbers in a population are controlled around a mean level over a number of generations and second, how this mean level is determined. Studies on the dynamics of insect populations leading to the construction

of life tables are frequently confined to a particular location where the fecundity and mortality of successive generations are estimated^{1–3}, and this approach emphasises the first aspect. Varley⁴ appreciated the value of obtaining permanent traces of the insects as they passed from one instar to another (galls, emergence holes, exuviae), since such direct counts of the numbers entering an instar avoided the time-consuming and inaccurate process of integrating successive estimates of population size⁴. The traces are analogous to a natural accumulative trap. Beaver⁵ and Danks⁶ used this 'trace' method for bark beetles and various solitary Aculeata. Freeman⁷ extended the trace method to accumulated generations at each of several sites, an approach which emphasises the second aspect of population dynamics.

Our studies in Jamaica on the population dynamics of solitary aculeates commenced with the wasp *Sceliphron assimile* Dahlbom^{7–9}. This sphecid builds mud cells, in each of which a single egg is laid⁷. Thus the number of cells constructed by a female constitutes a long-lasting record of her fecundity. Instars which die within cells constitute a similar record of mortality, whereas emergence holes simply measure adults emerged. Moreover, the predators, parasites and competitors can be identified by traces of their entry into or exit from the cells, or by the cast exuviae or faecal pellets within them. The traces thus represent data accumulated for many successive generations⁸.

In temperate countries the alternation of summer and winter tends to regulate the synchrony of generations, but in the tropics this is frequently not so and the concept of generation loses much of its value. Although *S. assimile* breeds at a reduced rate between January and March and some prepupae diapause, during the rest of the year about five to six overlapping generations occur. Disused cells accumulate with very small losses for at least seven years or some 40 such generations. Cells belonging to different generations or years cannot be accurately separated. Thus their study and dissection at any site provide data which although not generation(time)-specific are location(space or place)-specific, since we could not expect the pattern of fecundity and mortality relating to so many generations to be very different from any other equal or longer period. Note, however, that the variation of fecundity, mortality and migration with time cannot be assessed.

It is a relatively short task to dissect a large sample of cells from a particular locality, and from this to construct a retrospective place-specific life table. Many localities can be investigated and compared, and thus the spatial pattern in population dynamics can be more simply explored since the temporal one is suppressed. Also, life tables from many localities can be summed to produce one which is a species-specific character for an entire population and therefore of similar status to a human life table for a particular nation.

The major problem in this work is to relate the density of the disused cells at any site to that of the living animals on which mortality factors have been operating. The former are naturally the sum of the latter, but errors would arise if cells had been accruing at one site for a longer period than at another, or if the disappearance rate of disused cells was greater at one site than at another. The numbers of living and disused cells of *S. assimile* were correlated⁸ ($r = +0.96$), but errors can be further reduced by sampling at comparable sites—for example, rejecting sites where cells have been accruing for only a year or two. Although specifically unaccountable errors must be present, we estimated¹⁰ in *Pachodynerus nasidens* (Latr.) their magnitude to be about $\pm 20\%$, which is relatively small compared with the range of density of disused cells (about 1–1,000 m²).

Within the limitations of our method, density-dependent mortality with respect to place is well established in our

tropical aculeates. *Melittobia ?hawaiiensis* (Eulophidae), (formerly recorded as *M. chalybii* Ashmead) is the chief parasitoid of solitary Aculeata in Jamaica. The correlation coefficients of percentage mortality caused by it on the density of the following Aculeata were:

Sceliphron assimile: $r=+0.84$, $P<0.0001$ (ref. 8); *Zeta abdominale* (Drury), (= *Eumenes colona* Saussure): $r=+0.72$; $P<0.01$ (ref. 11); *Pachodynerus nasidens*: $r=+0.52$, $P<0.01$ (ref. 10). In *Zeta abdominale* predation by the miltgrammine fly *Amobia floridensis* Townsend was again density dependent, $r=+0.60$, $P<0.02$ (ref. 11). Developmental mortality was high at places where these species were usually dense. Since time is not accountable we are unable to detect delayed density dependence¹.

Since so many generations are being considered, mortality and fecundity must balance unless there is strong evidence that the species is becoming more (or less) numerous and/or extending (or contracting) its range. Even so they will tend to be equal. To analyse the situation further the k -value budget of Varley and Gradwell¹² which measures the $\log_{10}$ differences between successive population density estimates in a single generation, can be modified to suit the spatial approach. First, estimates of the density of any developmental instar are necessarily accrued so that the k values (here called $\hat{k}$ values) between them measure total mortality for a particular stage over several generations. Note that they are not mean k values ($\bar{k}$) since dense generations would over-contribute to them. Second, since over the period during which the cells are accruing, fecundity and mortality will be almost exactly equal, a cyclic budget returning to a constant value—say, at the egg stage—can be constructed:

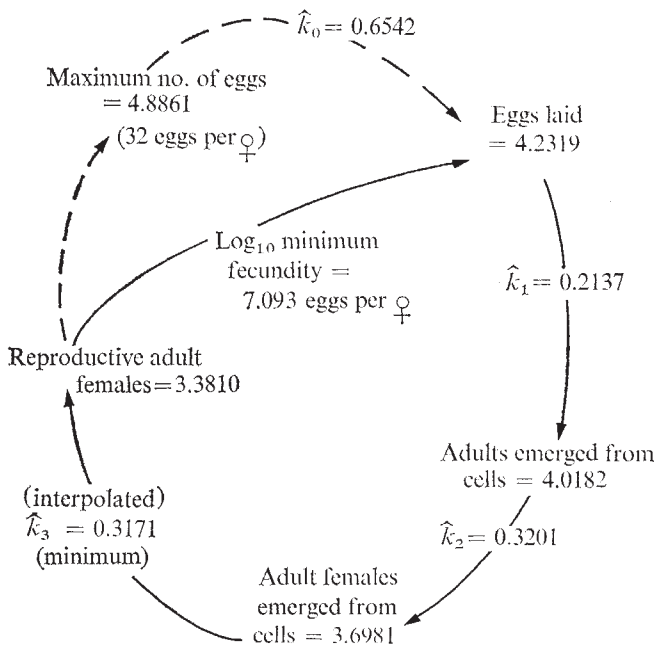


Fig. 1 A cyclic budget for *S. assimile* in Jamaica (see text). $\hat{k}_0$, Loss of fecundity; $\hat{k}_1$, developmental mortality; $\hat{k}_2$, loss of males; $\hat{k}_3$, pre-reproductive mortality/migration of females.

Figure 1 traces the fate of 17,059 ($\log_{10}=4.2319$) eggs laid during some 40 generations. The sex ratio was equivalent to about 47.8% females, and the remaining males are considered as a 'loss' to the species and therefore equivalent to a mortality factor. The pathway of maximum fecundity (broken line) would occur if all reproductive females laid the maximum number of eggs recorded for a single female. Minimum fecundity is the average number of cells per nest in Jamaica corrected for those cells in which no egg was laid⁷. Observations on marked females indicate that

fecundity achieved was 10–15 eggs per reproductive female. $\hat{k}_0$ measures the maximum loss of fecundity due to the death of reproductive females before they have laid all their eggs. The budget has the important property that any missing $\hat{k}$ value (here $\hat{k}_3$) can be interpolated. Thus, if achieved fecundity was 12.5, $\hat{k}_3$ would rise to 0.5631 and $\hat{k}_0$ would fall to 0.4082.

Budgets for specific localities will contain an immigration–emigration factor and this will usually occur in the preproductive adult stage; but the one above is for Jamaica as a whole, and therefore we expect this factor to be small. Budgets for specific localities provide quantitative data on how population levels are achieved and their comparison may enable factors causing the specific population level to be identified, but they cannot reveal the mechanism of control. Budgets for entire populations enable inferences about the life strategy of a particular species to be made.

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B. E. FREEMAN

Department of Zoology,
PO Box 12,
University of the West Indies,
Mona, Kingston, Jamaica

Received November 7, 1975; accepted January 20, 1976.

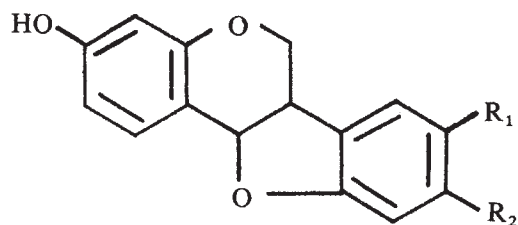
- 1 Varley, G. C., *J. anim. Ecol.*, **16**, 139–87 (1947).
- 2 Richards, O. W., and Waloff, N., *Phil. Trans. R. Soc.*, **B224**, 205–57 (1961).
- 3 Varley, G. C., and Gradwell, G. R., *Insect Abundance* (edit. by Southwood, T. R. E.), *Symp. R. Ent. Soc. Lond.*, **4**, 132–42 (1968).
- 4 Southwood, T. R. E., *Ecological Methods* (Methuen, London, 1966).
- 5 Peaver, R. A., *J. anim. Ecol.*, **35**, 27–41 (1966).
- 6 Danks, H. V., *J. anim. Ecol.*, **40**, 79–82 (1971).
- 7 Freeman, B. E., *J. anim. Ecol.*, **42**, 173–182 (1973).
- 8 Freeman, B. E., and Parnell, J. R., *J. anim. Ecol.*, **42**, 779–784 (1973).
- 9 Freeman, B. E., *Carib. J. Sci.*, **14**, 115–124 (1974).
- 10 Freeman, B. E., and Jayasingh, D. B., *Oikos*, **26**, 86–91 (1975).
- 11 Freeman, B. E., and Taffe, C. A., *Oikos*, **25**, 388–394 (1974).
- 12 Varley, G. C., and Gradwell, G. R., *J. anim. Ecol.*, **29**, 399–401 (1960).

Phytoalexin induction as a new dynamic approach to the study of systematic relationships among higher plants

It is well established that many higher plants respond to microbial invasion by the *de novo* production of organic substances called phytoalexins^{1,2}. These compounds are absent from healthy plants and are induced by the attacking microorganisms. Although the role of phytoalexins in disease resistance is not yet entirely clear, considerable evidence suggests that they are of importance in the protection of higher plants from fungal colonisation. Although few surveys have been attempted, there is clearly a taxonomic element in phytoalexin biosynthesis, in that different plant families accumulate chemically different types of compounds³. Thus, the Leguminosae in general produce isoflavonoids, the Solanaceae diterpenes, the Compositae polyacetylenes and so on²; anomalies are rare, for example, the furanoacetylene, wyerone acid, from *Vicia faba* (Leguminosae)⁴. As lesser variations also occur within these families, there is the clear possibility of using phytoalexin induction as a tool in taxonomic studies. We report here the first successful application of this technique to the problems of classification at the generic and species level in the Leguminosae.

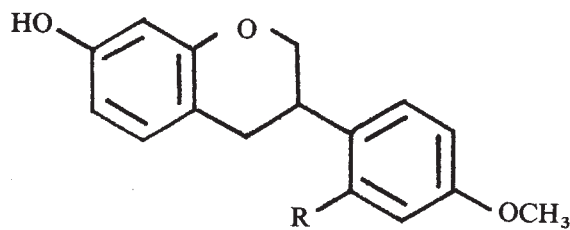
The first genus studied was *Trigonella*, a group of some 70 annual species, the best known member being the spice plant, *T. foenum-graecum*. *Trigonella* is a taxonomically 'difficult' genus because it merges into the closely related *Medicago* and *Melilotus*⁵; indeed, some of its species have at one time or another been variously classified under these other two genera. Young plants were grown from authenticated seed samples of 35 *Trigonella* species. Phytoalexins

were obtained from detached leaves using the drop-diffusate technique⁶; conidial suspensions (about 5×10^4 spores ml^{-1}) of the non-pathogenic fungus, *Helminthosporium carbonum* in 0.05% aqueous Tween-20 were used as the phytoalexin inducer⁷. Control leaves were inoculated with deionised water containing Tween-20. After 48 h of incubation, the droplets (diffusate) into which the phytoalexin had diffused, were collected and the antifungal component(s) isolated and identified by direct comparison (thin-layer chromatography, ultraviolet and mass spectroscopy) with authentic samples. The fungitoxicity of compounds I, II, III and IV was determined in tests against the mycelial growth of *H. carbonum*.



I: $R_1 = \text{H}$; $R_2 = \text{OCH}_3$

II: $R_1 = R_2 = \text{O}-\text{CH}_2-\text{O}$



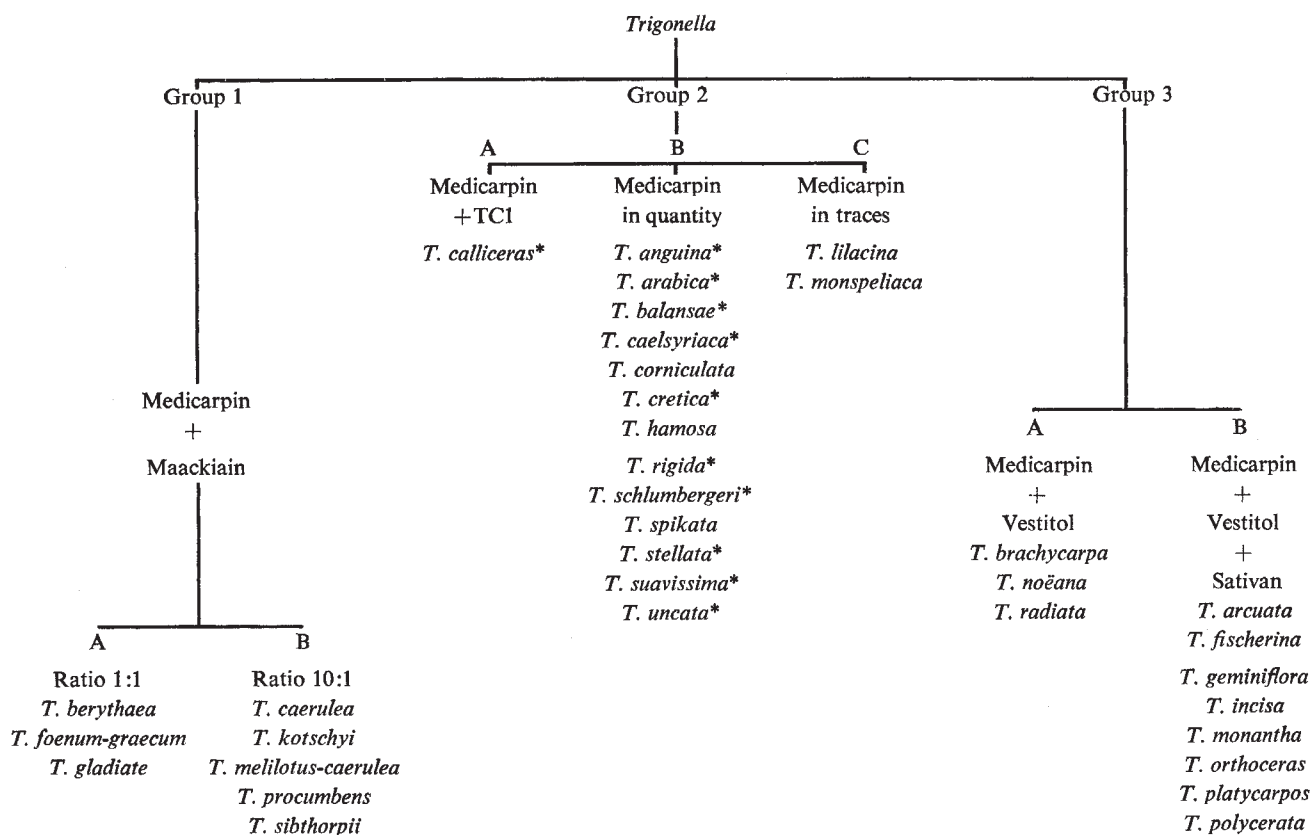
III: $R = \text{OH}$

IV: $R = \text{OCH}_3$

All 35 species produced one or more of five chemically related phytoalexins. Two of these were the known pterocarpan, medicarpin I and maackiain II while a third (compound TC-1) was provisionally identified as a new tri- or tetrahydroxy-pterocarpan. The others were the known isoflavans, vestitol III and sativan IV. All five compounds were absent from the control diffusates. Traces of three pterocarpan precursors, namely the isoflavone formononetin, the flavanone liquiritigenin and the chalcone isoliquiritigenin accompanied the above phytoalexins in a few species. The results of surveying all 35 species clearly indicate that *Trigonella* can be divided into three broad groups (Fig. 1); group 1, with medicarpin and maackiain; group 2, with medicarpin alone; and group 3, with medicarpin and vestitol. These groups were also divisible into several subgroups (Fig. 1). Thus, within the eight species of group 1, three produce medicarpin and maackiain in equal amounts, whereas the other five produce much more of the former than of the latter. Similarly in group 3, there is a division between the three species which synthesise medicarpin and vestitol and the eight which also produce sativan.

That phytoalexin studies allow *Trigonella* species to be placed within groups which are systematically meaningful is apparent from supporting morphological and chemical evidence. In particular, many species from group 2 show some morphological resemblance to *Melilotus*, a genus characterised by the production of medicarpin. Furthermore, a special chemical feature of *Melilotus*, namely the ability to release coumarin when tissue is macerated⁸ is present in most (11 of 16) of the *Trigonella* species in this group (Fig. 1) but is otherwise absent from the genus. Also, the 11 species of group 3 have morphological affinities with members of *Medicago*; again there is a parallel in phytoalexin synthesis in that *Medicago* species generally produce those compounds characteristic of the third *Trigonella* group. It is perhaps significant that taxonomists have made several attempts to move one member of group 3, namely *T. platycarpus*, into *Medicago*⁹.

Fig. 1 Groupings of *Trigonella* species based on their phytoalexin production. *Coumarin producer.



The above results also have some bearing on evolutionary relationships between these taxa, in that several of the phytoalexins can be placed in a biogenetically developing series. Thus, medicarpin might be converted to vestitol, from which sativan could then be obtained by methylation. Production of maackiain is an evolutionary step of some significance as this chemically 'advanced' pterocarpan cannot be obtained directly from medicarpin¹⁰. At the same time it should be noted that both isoflavans (III and IV) are considerably more antifungal than are either of the pterocarpanes. On this basis then, the three *Trigonella* groups fall into a series of increasing chemical advancement: group 2 → group 1 → group 3.

We report the present results because we believe that phytoalexin studies are generally applicable to systematic problems in angiosperms. We have successfully induced phytoalexin synthesis in twelve other leguminous genera and the results will be reported in detail elsewhere. Several known and new phytoalexins have been encountered in these genera, including resveratrol—a simple stilbene from groundnuts (*Arachis hypogaea*). Finally, we believe that phytoalexin surveys are economically important in that new naturally occurring compounds of potential value to crop protection may well be revealed.

JOHN L. INGHAM
JEFFREY B. HARBORNE

Phytochemical Unit,
Plant Science Laboratories,
University of Reading,
Whiteknights, Reading RG6 2AS, UK

Received December 18, 1975; accepted January 12, 1976.

- ¹ Deverall, B. J., in *Phytochemical Ecology* (edit. by Harborne, J. B.), 217–233 (Academic, London, New York, 1972).
- ² Ingham, J. L., *Bot. Rev.*, **38**, 343–424 (1972).
- ³ Harborne, J. B., in *Chemotaxonomy of the Leguminosae* (edit. by Harborne, J. B., Boulter, D., and Turner, B. L.), 275–278 (Academic, London, New York, 1971).
- ⁴ Letcher, R. M., Widdowson, D. A., Deverall, B. J., and Mansfield, J. W., *Phytochemistry*, **9**, 249–252 (1970).
- ⁵ Stevenson, G. A., *Can. J. Pl. Sci.*, **49**, 1–20 (1969).
- ⁶ Higgins, V. J., and Millar, R. L., *Phytopathology*, **58**, 1377–1383 (1968).
- ⁷ Ingham, J. L., *Nature*, **242**, 126–126 (1973).
- ⁸ Gorz, H. J., and Haskins, F. A., *Crop Sci.*, **4**, 193–196 (1964).
- ⁹ Baum, B. R., *Can. J. Bot.*, **46**, 741–749 (1968).
- ¹⁰ Dewick, P. M., *J. Chem. Soc. Commun.*, 656–658 (1975).

Evidence of hormonal control of ovulation in tsetse flies

ODHIAMBO¹ and Saunders and Dodd² reported that the first fully developed egg in the tsetse fly (*Glossina pallidipes*¹ and *G. morsitans*²) is not released from the ovary into the uterus until the female has had mating experience. They observed that the component of the mating act causing ovulation was not insemination, and suggested that a mechanical factor is responsible for the initiation of ovulation². It is difficult to see how a strictly nervous mechanism could account for the time lag between mating and ovulation (females mated 2–3 d after emergence usually ovulate 6–7 d later). Foster³ found that the ablation of median neurosecretory cells (MNC) of the pars intercerebralis almost always inhibited ovulation in *G. austeni*. He noted, however, that surgical brain trauma also inhibited ovulation in control flies. Ejezie and Davey⁴ found that the MNC undergo cyclic changes of net synthesis and release in *G. austeni* and attempted to correlate these phenomena with ovulation and larviposition. We describe here investigations of the control mechanism of ovulation in *G. morsitans morsitans*.

In our laboratory (24.5 ± 0.5 °C, 65 ± 5% relative humidity, 12 h light and 12 h dark and 100 lx at the level of experimental flies), females (fed on rabbit ears 6 d a week) are normally mated 2–3 d after emergence and ovulate 5–7 d later when they possess the first fully

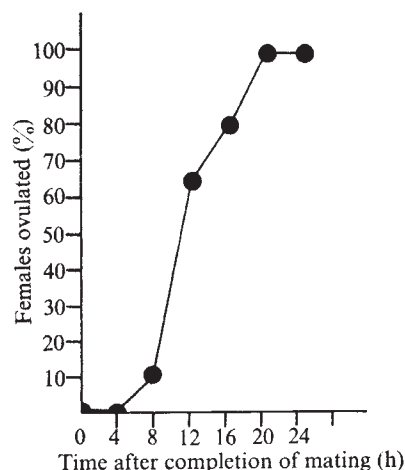


Fig. 1 Pattern of ovulation in delayed mated females. They were allowed to mate 12 d after emergence. Groups of 20 females were dissected 0, 4, 8, 12, 16, 20 and 24 h after termination of mating to determine whether ovulation had occurred.

chorionated egg. But we found that if the females were prevented from mating until their first egg was fully chorionated (11–12 d after emergence, to be certain) and then were allowed to mate ('delayed' mated), they ovulated within 24 h of termination of copulation (Fig. 1).

A male could inseminate a female (while ovulated subsequently) when allowed to mate uninterrupted for a prolonged period—about 60 min. But brief copulatory acts of 20 min or less did not result in insemination or ovulation. To obtain conclusive data on the relationship of mating, insemination and spermatophore formation to ovulation, we allowed the female to experience multiple interrupted (manually separated) matings of shorter than normal duration (10–20 min) with several different males consecutively, which allowed her to accumulate a prolonged mating experience but prevented her from receiving sperm or sufficient male accessory reproductive gland (AG)

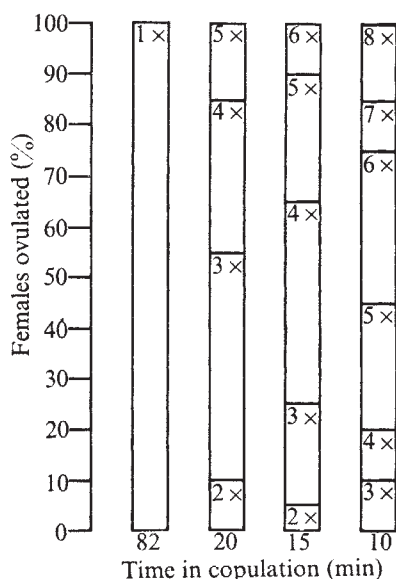


Fig. 2 Pattern of ovulation in females that experienced consecutive multiple matings of shorter than normal duration with fresh unmated males. Females (12 d old) were allowed to mate consecutively with two (2x) to eight (8x) males for 10, 15 or 20 min each by manually terminating each mating and allowing each female 30–100 min in copulation. Control females were mated with only one male (1x) and were not interrupted. Ovulation was checked 24 h after the last mating was terminated. Spermathecae were checked routinely to record any insemination. Each percentage is based on observations on 20 females.

secretion to form a spermatophore. Ovulation occurred in all females which spent 80–100 min copulating in several brief 'sterile' matings but the rate of ovulation declined with the decrease in copulation time (Fig. 2). Further observations of interrupted matings revealed that males could transfer some AG secretion during 10 min in copulation (unpublished work). To eliminate the effect of male AG secretion on ovulation, we removed the paired male AG, and, in some cases, the proximal part of the ejaculatory duct, and allowed the operated males to copulate with the virgin females. Twelve females which mated with the operated males for an average of 57 min ovulated, but five that mated for an average of 25 min did not.

There is no evidence of a male contact pheromone involved in induction of ovulation, as ovulation was demonstrated in virgin females which had no contact with males but into whose uteri one or two glass beads of about 300 μ m diameter (about half the size of a spermatophore) had been inserted through the vaginal opening. A comparable effect is the sensory perception of artificial stimulation of the reproductive tract which results in ovulation in some vertebrates⁵.

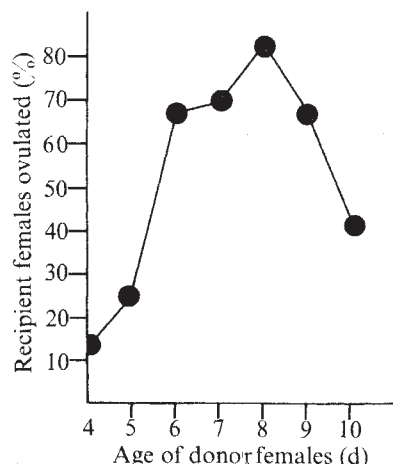


Fig. 3 Pattern of ovulation in virgin females after receiving haemolymph from the mated females of indicated age. Donor females were mated when 3 d old. Virgin recipients ($n = 15-20$) were 11–12 d old when they received haemolymph. Control virgin recipients ($n = 10$) received haemolymph from virgin donors. Ovulation was checked 48 h after the injection. Ovulation in controls = 0%.

We also removed the corpus allatum (CA) and corpus allatum-corpora cardiacum (CACC) of the female before or immediately after completion of mating. In either mating status the removal of CACC inhibited ovulation. Allatectomy of 9–12-d-old females did not interfere with the first ovulation.

Pursuing a different line of investigation, we induced ovulation in physiologically mature virgin females by injecting each of them with 0.5–0.8 μ l haemolymph taken from 4–10-d-old mated (mated when 3 d old) females. Preliminary results show a correlation between the age of donor females at the time of transfusion and the percentage of recipients ($n = 15-20$) showing ovulation (Fig. 3). Haemolymph from the virgin donors did not result in ovulation in virgin recipients ($n = 10$).

We draw the following conclusions from these observations. (1) Ovulation is dependent on the physical component of copulation and (2) is independent of insemination and the transfer of male AG secretion or seminal fluid. (3) The duration of the mating act is critical in inducing ovulation. (4) The CC is important in the regulation of ovulation; (5) a blood-borne factor in the mated female is ultimately responsible for induction of ovulation, and

(6) ovarian development itself may have a role in the control mechanism.

Our findings, together with earlier observations¹⁻⁴, suggest that afferent nervous impulses resulting from prolonged mating, and probably those resulting later from ovarian distension due to the presence of a fully developed oocyte, activate the MNC to release an ovulation-stimulating hormone into the haemolymph, probably through the CC. We suggest that this hormone ultimately induces ovulation.

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M. F. B. CHAUDHURY
T. S. DHADIALLA

International Centre of
Insect Physiology and Ecology,
PO Box 30772,
Nairobi, Kenya

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- ¹ Odhiambo, T. R., *J. exp. Zool.*, **177**, 447–454 (1971).
- ² Saunders, D. S., and Dodd, C. W. S., *J. Insect Physiol.*, **18**, 187–198 (1972).
- ³ Foster, W. A., *Bull. ent. Res.*, **483**–493 (1974).
- ⁴ Ejaz, G. C., and Davey, K. G., *Bull. ent. Res.*, **64**, 247–256 (1974).
- ⁵ Porter, R. W., Cavanaugh, E. B., Critchlow, B. V., and Sawyer, C. H., *Am. J. Physiol.*, **189**, 145–151 (1957).

Growth regulation in chimaeras between large and small mice

AGGREGATION chimaeras between strains of mice differing in body size may be used to determine whether the regulation of growth is determined by the relative proportions of cells deriving from the constituent strains. Should this be so, several questions arise; is growth regulated by the proportions in the body as a whole, or is the cellular composition of particular organs (or tissues) more critical? If critical organs or tissues are established, is the regulation then a systemic property, or does the response of other organs and tissues depend further on their own cellular composition? We describe here preliminary findings which indicate the answers to some of these questions and suggest ways of answering the others.

Aggregation chimaeras were made, by methods described previously¹, between mice from strains selected for large (L) and small (S) body size, and between L and unselected control (C) mice; the history of the strains is given in ref. 2. Some C $\leftrightarrow$ C chimaeras are included for comparison. Fifty overt chimaeras, all from C-strain foster mothers, were distributed among the three classes in the numbers shown in Table 1. Overt chimaerism was detected by marking the constituent strains with contrasting coat colours, either albino or coloured. Coat colours and body size were used reciprocally in chimaeras between L and S and since there was no difference between the reciprocals, they were pooled. In chimaeras between L and C, the latter was always the albino component. The proportion of albino in the coat was scored as described in ref. 3. We do not include data from successful aggregations which later resulted in single coat colours, but those mice ruled out any general tendency for L cells to outgrow S cells, or vice versa. This is supported by the fact that the mean proportion of L in the coat, in chimaeras both with S and with C, is close to 50% (Table 1). All data reported here were recorded when the mice were 6 weeks old. All female body weights were adjusted to male equivalents, males being 20% heavier in these strains. The weights of the constituent strains were adjusted further by regression to litter sizes equal to those in which the chimaeras were born, the weights thus seeming greater than those published earlier².

Examination of the data revealed a positive regression of body weight on the proportion of the L genotype in the variegation score of the coat (Fig. 1), the corresponding correlations being 0.51 and 0.70 in S $\leftrightarrow$ L and C $\leftrightarrow$ L

chimaeras, respectively. Cumulatively, there is no doubt about the significance of these regressions, and two conclusions follow. First, body size is linearly and directly proportional to the cellular composition of the coat of the chimaera. Second, since melanocytes themselves can hardly be determinants of growth, the observed correlation must reflect a fundamental correlation between the proportions of melanocytes in the coat and the corresponding proportions of L and S cells in whatever tissue(s) regulate growth.

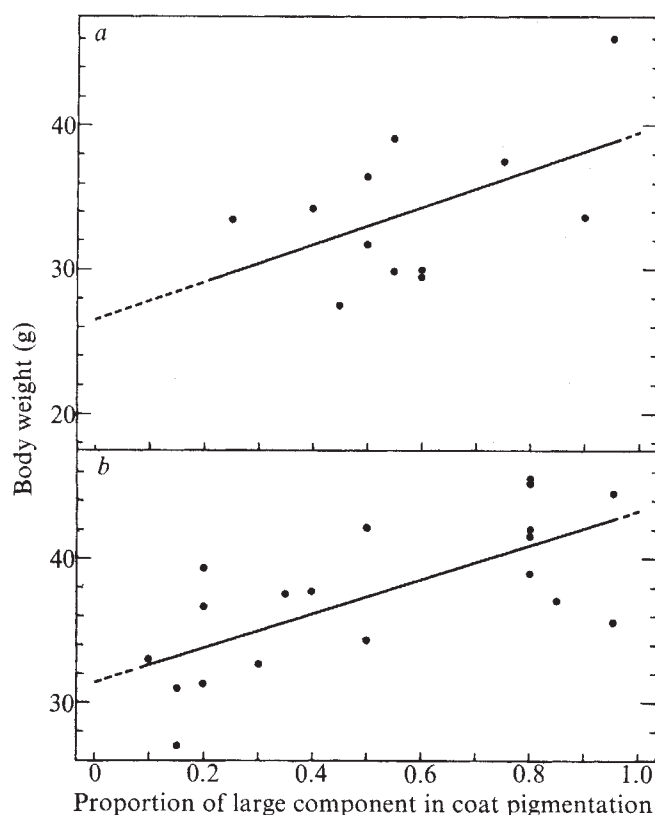


Fig. 1 Relationship between body weight and proportion of L component in coat pigmentation. Values of regression coefficients: in $S \leftrightarrow L$ (a), 13.0 ± 6.9 ; in $C \leftrightarrow L$ (b), 11.7 ± 2.9 .

A critical observation from Table 1 is that $S \leftrightarrow L$ and $C \leftrightarrow L$ chimaeras are 2.5 times more variable in weight than $C \leftrightarrow C$ chimaeras, whose own variance is about the expected normal value. A source of variance in chimaeras between different strains, not present in other mice, is the variation between individuals in the proportions of the two cell types. For coat colour, this proportion varied between 0 and 1, and covered most of that range even if the single colours were excluded. If the same kind of variance in proportion applies to weight-controlling tissue, the inflated variance of weight is readily explicable. It is therefore important to estimate the variance of the proportions of cells in the weight-controlling tissue.

We now define the 'chimaeric genotype' (G) for growth in terms of the proportions of the two types of cells. Let

$$G = PL + (1 - P)S$$

where P is the proportion of large cells (L), leaving $(1 - P)$ small cells (S). In the other class of chimaeras, C is substituted for S. On rearrangement, this gives

$$G = S + (L - S)P = S + DP$$

where D is the difference in weight between the constituent

strains. G is thus a linear function of P , as implied by Fig. 1. Since S , for present purposes, is a constant,

$$\text{var}(G) = D^2 \text{var}(P)$$

where $\text{var}(G)$ is the variance of G , and so on. Trivial reorganisation gives an expression for $\text{var}(P)$ —the variance of proportions of cells in the growth-controlling tissue. D is directly observable and $\text{var}(G)$ may be estimated, at least roughly, as follows. The variance in body weight of the $C \leftrightarrow C$ chimaeras does not contain any $\text{var}(G)$, by definition, since G was defined in terms of the proportions of differing cell types; however, the variance of the $C \leftrightarrow C$ chimaeras will reflect all other sources of variation. Subtracting the variance in body weight of $C \leftrightarrow C$ chimaeras from that of the $S \leftrightarrow L$ chimaeras therefore provides a direct estimate of $\text{var}(G)$ appropriate to that class. All the necessary data are available from Table 1, and using the expression derived above, we obtain estimates of 0.046 and 0.190 for $\text{var}(P)$ in $S \leftrightarrow L$ and $C \leftrightarrow L$ chimaeras, respectively. Viewed slightly differently, these are the variances in the cellular composition of the growth-controlling tissue that would be necessary to explain the increased variance in weight found in chimaeras between different strains. Although we make no claims for the numerical accuracy of these estimates of $\text{var}(P)$, the values found seem to be reasonable, and they correspond closely to the variance in the proportions of melanocytes, estimated from the coat by direct observation (see Table 1).

Table 1 Body weights and proportion of L component in coats

Constituent lines	L	C	S
Adjusted weights (g)	36.7	27.3	18.3
Chimaera type	$S \leftrightarrow L$	$C \leftrightarrow L$	$C \leftrightarrow C$
n	12	19	19
Coat: proportion L	0.58	0.52	(0.28)*
variance	0.040	0.096	(0.075)*
Weight (g): mean	34.1	37.5	28.2
variance	25.7	26.8	10.0

* These values refer to the proportion of albino in coat.

Our results therefore establish that growth in chimaeras is a direct function of the proportions of cells from the two constituent strains, which in turn implies that growth regulation resides in the cellular properties of certain organs or tissues. Further, from consideration of the variances of the proportions of cells, it seems that the system of growth regulation needs be neither more complicated nor more extensive than that controlling the distribution of melanocytes in the skin. This indicates that the organs or tissues governing growth control may eventually be located, and to that aim we are marking constituent strains with variants of an enzyme that can be assayed quantitatively. The higher the correlation between body weight and proportion of L cells in various tissues, the more important will that tissue be in regulating growth. Equally, the systemic effect deriving from such tissue can be examined against the cellular composition of target organs, to see whether there is any local autonomy of size regulation.

R. C. ROBERTS
D. S. FALCONER
PATRICIA BOWMAN
I. K. GAULD

ARC Unit of Animal Genetics,
Institute of Animal Genetics,
West Mains Road,
Edinburgh EH9 3JN, UK

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- 1 Bowman, P., and McLaren, A., *J. Embryol. exp. Morph.*, **23**, 693–704 (1970).
- 2 Falconer, D. S., *Genet. Res.*, **22**, 291–321 (1973).
- 3 Falconer, D. S., and Isaacson, J. H., *Genet. Res.*, **20**, 291–316 (1972).

Evidence for a new diffusible element of mating pheromones in yeast

THE yeast *Saccharomyces cerevisiae* produces pheromones which seem to participate in the mating process^{1,2}. Each of the two haploid mating types (a or α) produces a diffusible factor (called a factor or α factor, respectively) which causes cells of the opposite mating type to be retarded in the G_1 period of the cell division cycle³⁻⁶. The pheromones thus act to synchronise the cell cycles of mating cells before the formation of the diploid zygote. By a variation of the original assay for yeast mating pheromones^{8,9}, we have observed that cells of mating type a produce a new pheromone which inhibits action of the α factor.

The standard assay for the α factor requires the placing isolated a cells ('assay cells') near a source of α factor, for example, a heavy streak of α cells^{8,9}. The α cells are unable to initiate new rounds of budding² and instead undergo the α -factor response, a characteristic shape change (see Fig. 1c, d and e). Continued exposure to α factor results in the further elongation of the a cell for at least 12 h, at which time cells furthest from the source of the α factor resume budding (ref. 4, and R. Chan, L. Hartwell and T. Manney, personal communication).

When a dense streak of a cells is placed between the α cells and a -assay cells (Fig. 2a), no α -factor response is observed in the a -assay cells (although the response is seen within the streak of a cells). Figure 1 shows the response of a -assay cells with (Fig. 1a) and without (Fig. 1e) interposed cells. The interposed cells therefore exhibit a barrier to the α -factor response and are said to have the Bar⁺ phenotype. To determine whether the barrier effect is controlled by the mating type locus, we have examined the Bar phenotype of a number of different a strains, a/α strains, and two classes of α mutants (ste^-) which do not produce the α factor (ref. 7 and J.M.H. and I.H., unpublished). All of the a strains tested here are Bar⁺, and all the a/α strains are Bar⁻. Although the barrier phenotypes of the a strains and the a/α strains are clearly different, it has been observed that assay cells in the presence of an a/α barrier recover from α -factor inhibition more quickly than controls where no barrier is present. This may

indicate the partial expression of the Bar function in a/α diploids. (Analogous observations have been made by T. R. Manney (personal communication).) Interestingly, the α strains carrying ste^- mutations inseparable from the mating type locus (classes 1 and 2) differ in their Bar phenotype. It is thus clear that the mating type locus itself regulates the barrier effect.

To determine further the relationship between the barrier effect and genes involved in mating, we examined the Bar phenotype of a cells carrying mutations which block mating (ste^-). Six of the $a ste^-$ are Bar⁺, and three are Bar⁻ (see Fig. 1b and c). The barrier effect therefore seems to be under the control of genes essential to mating. Since all of the $a ste^-$ mutants tested were insensitive to the α factor (ref. 7, and unpublished), there is no correlation between the Bar phenotype and α -factor response. The sterile mutant of class 10 behaves in some respects, including its Bar phenotype, as an a mutant (see Table 1). It should be noted that the existence of Bar⁻ strains (a/α , αste^- , and some $a ste^-$) argues against the notion that the barrier effect is attributable to a nonspecific effect of the growth of cells in the barrier streak (Table 1).

The barrier effect could involve the adsorption of the α factor by the a cells within the barrier streak. Another possibility (not exclusive of the first) is that the barrier cells produce a 'diffusible barrier', that is, a diffusible inhibitor. To test that possibility, we rearranged the geometry of the standard α -factor assay so that the a -assay cells were placed between heavy streaks of α and a cells (the 'N technique', see Fig. 2b). Under such an arrangement, the assay cells furthest from the a streak responded to α factor, whereas those closest to the a streak did not. The presence of the a streak was not essential for the inhibition of the α -factor response. Rather, a cells seem to excrete an inhibitor into the agar. This diffusible effect can be seen by allowing a streak of a cells to grow on an agar slab for 6 h at 30 °C. The a cells should then be removed by cutting the agar (see Fig. 2b). The standard α -factor assay should then be carried out on the slab thus obtained using a -assay cells at varying distances from the α cells. As controls, the assay should be carried out in parallel on untreated agar, and on agar 'treated' by growth of an $a ste^-$ strain which is Bar⁻ (strain S111). As before, the a cells nearest to the α cells will respond to the α factor in all

Table 1 Ability of different strains to exhibit barrier and diffusible effects on response to α factor.

Strain*	Mating type	Barrier effect	Diffusible effect	a factor†
39, 73, 75, 94, X5-39	a	+	+	NT
S107, S111	$a ste^-$	—	—	NT
S110	$a ste^-$	+	—	NT
S108	$a ste^-$	+	NT	NT
VAB2 (class 3), VW9 (class 7)	$a ste^-$	+	+	+
VAB1 (class 5), VU3 (class 15)	$a ste^-$	+	+	—
VY3 (class 13)	$a ste^-$	—	—	+
VC73 (class 10)	()‡	+	+	NT
VC2 (class 1)	αste^-	—	—	NT§
VB3 (class 1)	αste^-	—	NT	NT§
VP1 (class 1)	αste^-	+	NT	NT§
VN33 (class 2)	αste^-	—	—	NT§
X14, X16, X17	$a/\alpha ho/ho$	—	—	NT
X47	$a/\alpha HO/ho$	—	—	NT
X10-1B	$a/\alpha HO/HO$	—	—	NT

In most cases the a -assay cells were strain 73 ($a-ily3-X$) and the α -factor source cells were strain 70 ($\alpha thr3-10$).

*Strains 73, 75, 94, X5-39 and X10-1B are described in ref. 10. S107, S108, S110, and S111 are sterile mutants isolated from X5-39, and carry mutations $ste107$, 108, 110, and 111, respectively (unpublished). The V series sterile mutants are described in ref. 7.

†Ref. 7.

‡VC73 was isolated from an α parent and does not produce the α factor⁷. It mates with α cells 1% as efficiently as do wild-type a cells (unpublished).

§These mutants do not produce the α factor.

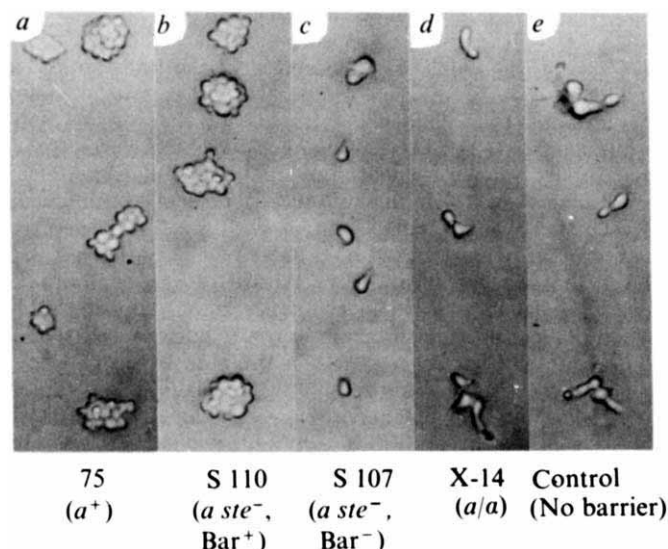


Fig. 1 The α -factor response by a -assay cells (strain 73) in five separate barrier tests photographed after 12 h incubation at 30 °C. The strain designations below each panel refer to the barrier cells. Arrangement as in Fig. 2a. Growth of assay cells in (a) and (b) is the same as in the absence of α cells (data not shown) and indicates the absence of α -factor activity. The production of morphologically aberrant cells in c, d, and e is the normal response to the α factor exhibited by α cells. The α -factor source was strain 70.

cases. The α cells further from the α streak, however, will not exhibit the α -factor response on agar treated previously with a cells. On untreated agar or on agar treated by the growth of the $a ste^-$ strain, all of the a -assay cells will exhibit the α -factor response. This indicates that a cells produce a diffusible inhibitor of the α -factor response.

To test whether the inhibitor acts on α cells (for example, on α -factor synthesis or release), we looked for inhibition in the absence α cells when the α factor was present in the agar. The α cells were grown on an agar slab (Fig. 2b) for 6 h, and were then removed by cutting away an agar slab. The slab was cut in half horizontally (Fig. 2b) to give slabs A and B. A dense streak of a cells was laid down on slab A, and a streak of $a ste^- Bar^-$ (S111) was laid down on slab B. The α -factor response by a -assay cells placed on slab A was considerably less than the response on slab B. The diffusible inhibitor can, therefore, act in the absence of α cells.

The relationship between the Bar^+ effect and the diffusible inhibitor was examined by testing a number of strains for the diffusible inhibitor by the N technique (Fig. 2b and Table 1). No Bar^- strains (a/a , $a ste^-$, or $a ste^-$) exhibited a diffusible effect. All the strains tested which showed a diffusible effect were Bar^+ but not vice versa. We observed one case in which a Bar^+ strain (S110) did not show the diffusible effect. Further analysis of this mutant and characterisation of more $a ste^-$ mutants will be necessary in order to determine the relationship between the barrier effect and the diffusible inhibitor.

Therefore, a cells are able to block in some manner the action of the α factor. The ability of strains to exhibit a barrier effect is controlled by the mating type locus and by genes essential for mating, suggesting that it may be a normal component of the mating process. The observation that class 1 sterile mutants differ in their Bar phenotypes provides further evidence for the complexity of the mating type locus. At least part of the barrier effect is mediated by a diffusible factor. This factor does not necessarily act on the synthesis or release of the α factor by α cells since its action is seen in the presence of the α factor.

The inhibitor, thus, may act directly on a cells to modify their response to the α factor itself. The production of the inhibitor does not require induction by the α factor. Finally, the inhibitor seems to be distinct from the α factor since some strains which are α -factor deficient produce the inhibitor and vice versa (Table 1).

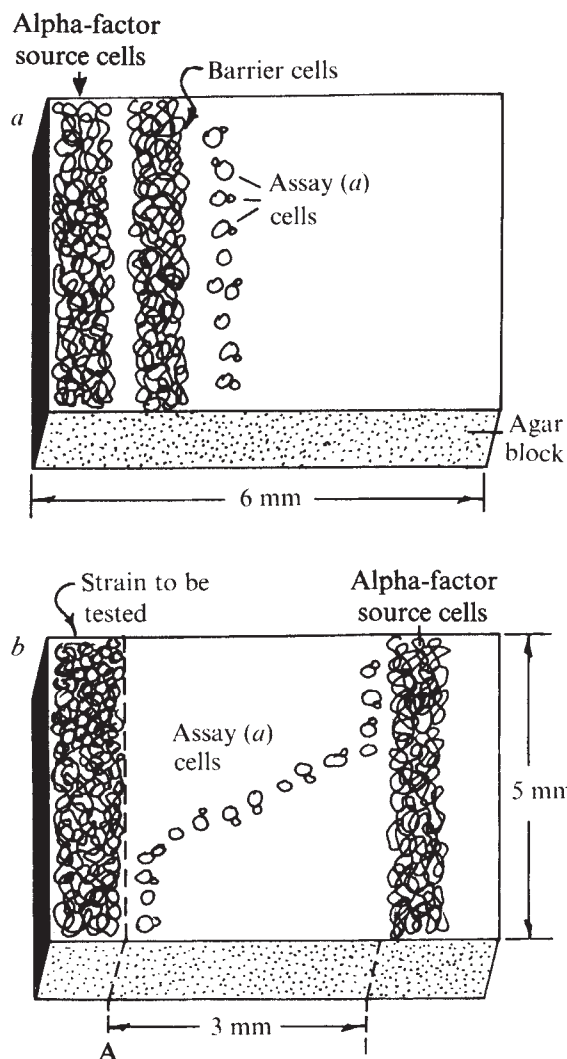


Fig. 2 Experimental arrangement for studying the barrier effect (a) and the diffusible inhibitor (b) of response to the α factor. Cells were pregrown overnight at 30 °C on YEPD agar (2% peptone, 1% yeast extract, 2% glucose, 2% agar). In the barrier test, α cells (α -factor source) and barrier cells were applied to blocks of dissection agar (2% peptone, 1% yeast extract, 4% glucose, 4% agar) in streaks containing approximately 10^7 cells. The blocks were placed on a glass coverslip and inverted over a dissection chamber. From 10 to 20 individual mating type a cells (a -assay cells) were then placed by micromanipulation on the agar in the position shown, 2 ± 0.2 mm from the source cells, and the chamber was sealed to prevent any loss of moisture. The growth rate and morphological characteristics of the cells were used as an assay of α -factor activity in the agar. The presence of α -factor activity in the agar distal to the barrier cells was indicated by distorted cell morphology (shmooing) and the absence of cell division by the assay cells after 12 h incubation at 30 °C (Bar phenotype; Fig. 1: c, d, e). The absence of α -factor activity was indicated by the ability of the assay cells to divide normally and form microcolonies (Bar^+ phenotype; Fig. 1: a and b). To determine whether strains produce a diffusible inhibitor of α -factor response, the streak of cells to be tested was applied to each agar block first as in arrangement b. After growth for 6 h at 30 °C, the section of agar containing this streak was cut away (along dotted line A in the diagram), and α -factor source cells and a -assay cells applied to the block as shown. The response of the assay cells was observed microscopically at 6, 12, and 24 h.

What would be the role of the barrier effect and diffusible α -factor inhibitor in the normal mating process? We offer three suggestions. First, the inhibition of α -factor response may be part of the mechanism by which a cells recover from an α -factor block⁴ and by which newly-formed a/α zygotes escape from inhibition by a and α factors in their environment. Second, successful mating between individual cells requires cell contact. The α factor can, however, act at a distance to inhibit the growth of a cells. The 'anti- α factor' activity may reduce the effective concentration of the α factor in the immediate environment of an a cell so that only the a cells very close to α cells will respond.

Third, the successful mating between populations of haploid cells requires equal numbers of cells of each mating type. In situations in which the number of a cells is greater than the number of α cells, mating would result in a small number of diploid cells and a large number of unmated a cells. If, however, the response to the α factor is blocked by a high concentration of the inhibitor, mating could be delayed until the further growth of α cells has occurred. The number of a and α cells would thus become comparable, and a greater fraction of the population could mate successfully.

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JAMES B. HICKS
IRA HERSKOWITZ

Department of Biology and
Institute of Molecular Biology,
University of Oregon,
Eugene, Oregon 97403

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¹ Mortimer, R. K., and Hawthorne, D. C., in *The Yeasts*, 1 (edit. by Rose, A. H., and Harrison, J. S.), 385-460 (Academic, New York, 1969).

² Bücking-Throm, E., Duntze, W., Manney, T. R., and Hartwell, L. H., *Expl Cell Res.*, **76**, 99-110 (1973).

³ Hartwell, L. H., *Expl Cell Res.*, **76**, 111-117 (1973).

⁴ Wilkinson, L. E., and Pringle, J. R., *Expl Cell Res.*, **89**, 175-187 (1974).

⁵ Scherer, G., Haag, G., and Duntze, W., *J. Bact.*, **119**, 386-393 (1974).

⁶ Hereford, L. M., and Hartwell, L. H., *J. molec. Biol.*, **84**, 445-461 (1974).

⁷ MacKay, V., and Manney, T. R., *Genetics*, **76**, 255-271 (1974); *Genetics*, **76**, 273-288 (1974).

⁸ Levi, J. D., *Nature*, **177**, 753 (1956).

⁹ Duntze, W., MacKay, V., and Manney, T. R., *Science*, **168**, 1472-1473 (1970).

¹⁰ Hicks, J. B., and Herskowitz, I., *Genetics* (in the press).

Regulation of the fibroblast cell cycle by serum

WHEN "normal" fibroblasts are deprived of serum they cease growing and accumulate in the G_1 or G_0 stage of the cell cycle¹. On restoration of serum, the cells begin to enter S phase quasi-synchronously after a well defined lag period which seems to be characteristic of the cell type. Following the studies of Todaro *et al.*², Bürk³, and Temin⁴, it is widely believed that exposure to serum for only a portion of this lag period is sufficient to commit the cells irreversibly to initiate a round of DNA replication. Thus, removal of the serum after only a few hours, and before the end of the lag period, does not affect subsequent DNA synthesis in a significant proportion of the population. It has been apparent, nevertheless, that the proportion of such "committed" cells depends on the duration of the serum pulse²⁻⁵. Indeed, the relationship between the length of exposure to serum and the proportion of committed cells is approximately linear with time and (with 3T3 cells) extends beyond the end of the lag phase itself (Fig. 1). Clearly, some cells which are stimulated to initiate DNA synthesis during continuous exposure to serum are not committed by the end of the lag period, since removal of

the serum at this point reduced the proportion of labelled cells, at 24 h, from 76 to 53% (Fig. 1b).

Experiments such as these have been interpreted as evidence that some cells require longer periods of mitogenic stimulation than others to become irreversibly committed to division⁶—a view which is apparently reinforced by the well known asynchrony with which cells begin DNA synthesis, even when the mitogen is present continuously. Recently, however, it has been shown⁷ that this asynchrony is not necessarily evidence of cellular heterogeneity but may be a consequence of the very nature of the commitment event itself⁷. In the presence of micromolar concentrations of metabolisable purines or purine nucleosides (which potentiate the serum response^{8,9}) the rate of entry into S phase increased very abruptly after the lag period, rapidly becoming first order⁷. Thereafter, a constant fraction of the G_1 population began DNA synthesis per unit time. This suggested that commitment was a random event occurring with a characteristic probability, as proposed earlier by Smith and Martin on other grounds¹⁰. The rate constant (or transition probability) for entry into S phase was found to be a function of the serum concentration, which means that this determines the rate of commitment rather than the number of committed cells. In the absence of exogenous purines, the rate constant increased slowly with time, but in their presence, the new value was attained abruptly with

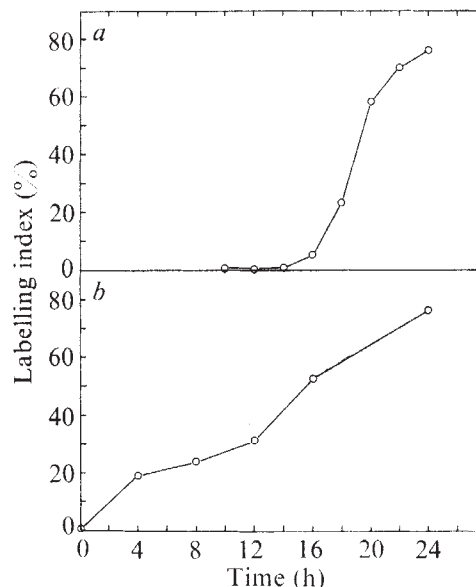
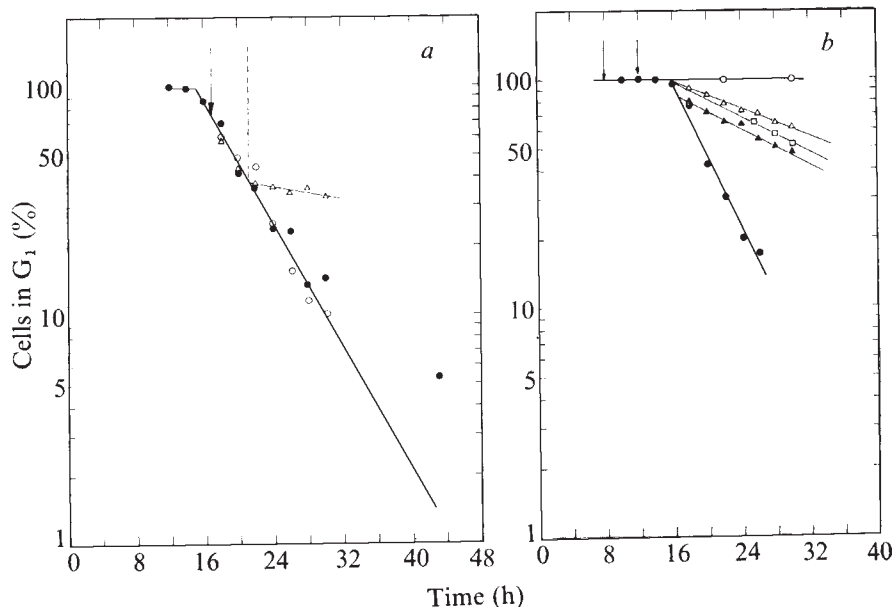


Fig. 1 *a*, Time course of entry into S phase after stimulation of quiescent 3T3 cells with 4% serum. Subconfluent, quiescent cultures of (Swiss) 3T3 cells¹³ were prepared by plating 3×10^4 cells on to 15-mm glass coverslips in each well of a Linbro multidish tray (Linbro 24-16-TC), in 1 ml of 0.5% calf serum in Vogt and Dulbecco's modified Eagle's medium¹⁴, followed by incubation at 37 °C for 3 d. Coverslips were then transferred to 3-cm plastic Petri dishes containing 2 ml of fresh medium and 4% calf serum (previously dialysed against 2×50 volumes of isotonic phosphate-buffered saline), together with inosine (25 μ M) and ³H-thymidine (1 μ Ci ml⁻¹; 3 μ M thymidine). With this ratio of medium volume to cell number, depletion or inactivation of serum activity^{15,16} is not significant during the subsequent course of the experiment. At the times indicated, pairs of coverslips were removed and processed for autoradiography as described previously⁷ using Kodak NTB3 nuclear track emulsion. After exposure for 2 weeks at 4 °C, autoradiographs were developed, stained with Geimsa solution and the proportion of cells with labelled nuclei determined. *b*, The effect of serum pulse length on the proportion of cells in S phase at 24 h. Quiescent 3T3 cells were stimulated with 4% dialysed calf serum in medium containing inosine (25 μ M) and ³H-thymidine (1 μ Ci ml⁻¹; 3 μ M thymidine). At the times indicated, pairs of coverslips were transferred to fresh medium containing 0.25% dialysed serum, but with the same concentrations of inosine and ³H-thymidine. After 24 h all coverslips were removed and prepared for autoradiography. The abscissa represents the duration of exposure to 4% serum.

Fig. 2 *a*, The effect of serum step-down on the rate of entry into S phase. Quiescent 3T3 cells were stimulated with 10% dialysed calf serum in the presence of inosine (25 μ M) and 3 H-thymidine (1 μ Ci ml $^{-1}$; 3 μ M thymidine). After 16 h, some coverslips were washed with warm (37 $^{\circ}$ C) serum-free Dulbecco's modified Eagle's medium and transferred to fresh medium containing the same concentration of inosine and 3 H-thymidine and either 10% dialysed serum ($\circ$) or 0.25% dialysed serum (Δ). Other coverslips remained untouched ($\bullet$). Pairs of coverslips from each set were removed at the times indicated and processed by autoradiography. The labelling index was determined and the proportion of cells remaining in G₁ obtained by subtraction, except for the point at 44 h which was calculated by counting the number of unlabelled cells per field and estimating the original cell density (that is, before cell divisions) from the preceding coverslips. *b*, As for (*a*) except that the highest serum concentration used was 4%, and the step-down to 0.25% made at either 8 h (Δ) or 12 h ($\blacktriangle$). $\circ$, 0.25%; $\square$, 1%; $\bullet$, 4%. For clarity, the early points for the 1% serum control ($\square$) have been omitted.



little evidence of passing through intermediate values. Once attained, the new value remained constant for at least a further 30 h (unpublished data). This suggested that the probability of initiating the cell cycle might reflect an equilibrium steady state in the rate of some cellular activity which in turn was controlled by the serum concentration. According to this interpretation, maintenance of the transition probability is regarded as a dynamic process dependent on the continuous presence of the growth stimulus, while much (and perhaps all) of the lag period is considered to be the time required to alter this equilibrium state. This hypothesis predicts that a reduction of the serum concentration at any point will be accompanied, some time later, by a decline in the rate of initiation.

Evidence in favour of this interpretation has been obtained by measurement of the proportion of cells remaining in G₁ at various times following stimulation. When the serum concentration was reduced from 10 to 0.25% at 16 h, the rate of initiation declined abruptly 5 h later (Fig. 2*a*). This decline was not the nonspecific result of manipulations involved in changing the medium but depended on the alteration of serum concentration. In other experiments, serum step-downs have been made after 12 h (Fig. 2*b*) or 20 h exposure (not shown): in each case the rate of initiation declined to a new steady state between 3 and 5 h later, regardless of how many cells had reached S phase at the time of the perturbation.

Following the step-down, the rate of entry into S phase does not decline to the very low value of the control (0.25% serum continuously), but remains elevated at a rate approximately equivalent to 1% serum. Whether this is the result of serum growth factors remaining absorbed to the cell surface in a manner that survives washing, or to some other reason, is not known. This residual rate of initiation shows some dependence, however, on the duration of the preceding serum pulse. When the step-down is made more than 5 h before the end of the lag phase, it is precisely this residual rate of initiation which seems to be responsible for the apparent "commitment" described by others following short serum pulses²⁻⁵ (Fig. 2*b*). As when serum is present continuously, the number of cells stimulated to begin DNA synthesis depends on the interval of time considered.

These experiments indicate that the apparent simplicity of the relationship between the length of exposure to serum and the proportion of committed cells (Fig. 1*b*) conceals a complex situation involving both the interval of observation and changes in the rate of initiation. There is no compelling evidence for subpopulations of hypersensitive cells poised,

to varying degrees, close to the commitment step and requiring only short serum stimulation. The experiments also suggest that commitment to DNA synthesis in 3T3 cells does not occur earlier than 5 h before S phase, regardless of when initiation actually takes place in each cell relative to serum addition. This must be so because otherwise it would not be possible to prevent the initiation of DNA synthesis in a committed cell by withdrawal of serum unless there were some additional serum-dependent step between commitment and the start of S phase. This possibility can be dismissed because the initial lag period between serum addition and the beginning of DNA synthesis—which, by definition must include such a hypothetical step—is completely independent of serum concentration^{4,7}.

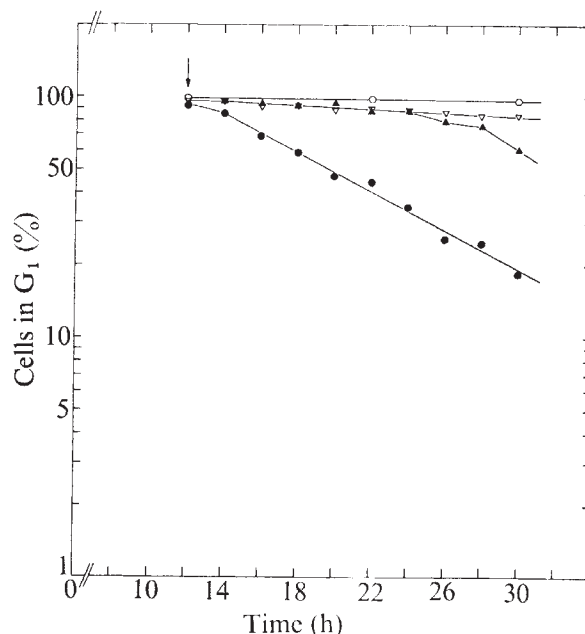


Fig. 3 The effect of serum step-up on the rate of entry into S phase. Quiescent 3T3 cells were stimulated with either 1% (∇) or 4% ($\bullet$) dialysed serum. After 12 h some of those coverslips which had been placed in 1% serum were transferred without washing to fresh medium containing 4% dialysed serum ($\blacktriangle$). ($\circ$, 0.25% serum). At the times indicated, pairs of coverslips from each set were removed and processed by autoradiography. The labelling index was determined and the proportion of cells remaining in G₁ obtained by subtraction. Inosine (25 μ M) and 3 H-thymidine (1 μ Ci ml $^{-1}$; 3 μ M thymidine) were present throughout.

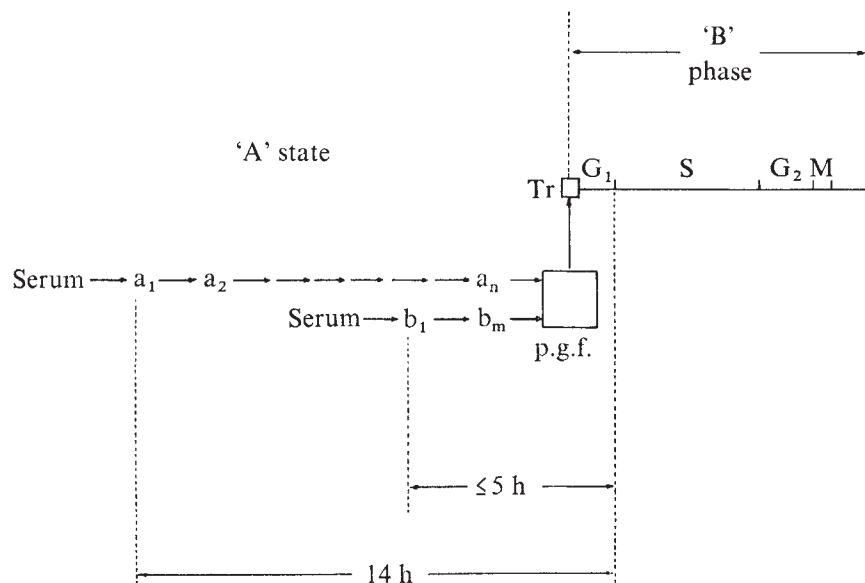


Fig. 4 A schematic view of the regulation of the cell cycle by serum. Serum concentration is considered to control the rate of at least two independent, convergent sequences of interconnecting, on-going processes (a or b). The point of convergence has been drawn, arbitrarily, at an hypothetical "probability generating function" (p.g.f.) postulated to activate a "trigger" (Tr) which, in turn, establishes the commitment to divide. Following commitment, the series of deterministic events leading up to mitosis have been labelled 'B' phase, after Smith and Martin¹⁰. All other processes are considered to take place in G_0 or 'A' state¹⁰, though these activities do not necessarily cease after commitment has occurred.

Although the commitment event cannot occur more than 5 h before S phase, however, the present data are not incompatible with an even closer coincidence with the beginning of S phase, as suggested recently by Rubin and Steiner¹¹, and by Smith and Martin¹².

In contrast to the effect of a serum step-down, which is manifest within 5 h, when the serum concentration is raised from a subsaturating level (1%) to a higher serum concentration (4%), no further increase in the rate of initiation is observed until after another 12–14-h lag (Fig. 3). This is the case whether the step-up is made after 12 h (Fig. 3) or 8 h, or from a higher initial serum concentration (not shown), and is thus independent of previous stimulation. Step-ups have not been made later than 12 h because continuation of such experiments beyond 30 h is complicated by the appearance of mitoses which render the cumulative labelling index less meaningful. For this reason, it is therefore not possible, at present, to prove rigorously that the final rate of initiation after step-up is identical to that produced by the higher serum concentration when present from the beginning. From the available data, however, this seems to be a reasonable supposition.

It is thus evident that the 14-h lag represents the time required to effect any increase in the probability of commitment, regardless of the initial value. Nevertheless, the transition probability decreases within 5 h on withdrawal of serum. This disparity suggests that serum controls at least two distinct cellular activities necessary for the maintenance of the transition probability. This is depicted schematically in Fig. 4 as two independent sequences of interconnecting processes, arbitrarily drawn to converge at the "probability generating function" (p.g.f.). Serum is considered to regulate the rate of the initial process in each sequence (a_1 or b_1) while the lag after either step-up or step-down is determined by the relaxation time for the respective sequences (a or b) as a whole to reach the new steady state. In the sense that each constituent process is viewed as ongoing (with serum concentration determining the rate), they should not be considered as events through which the cell must progress. Indeed, they should be regarded as outside of the cell cycle in G_0 (the 'A' state of Smith and Martin¹⁰), in so far as they occur before the commitment step. The value of the transition probability would seem to be set by the longest sequence, while the shorter is presumably concerned with some permissive function. It will be interesting to determine whether the processes a_1 and b_1 are regulated by different serum factors. The possible basis of the p.g.f. has been discussed pre-

viously¹²; further speculation is inappropriate here.

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R. F. BROOKS

*Molecular Biology Laboratory,
The Salk Institute,
Post Office Box 1809,
San Diego, California 92112*

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- ¹ Brooks, R. F., in *Structure and Function of Plasma Proteins*, 2 (edit by Allison, A. C.) (Plenum, London, in the press).
- ² Todaro, G. J., Lazar, G. K., and Green, H., *J. cell. comp. Physiol.*, **66**, 325–334 (1965).
- ³ Bürk, R. R., *Expl Cell Res.*, **63**, 309–316 (1970).
- ⁴ Temin, H. M., *J. cell. Physiol.*, **78**, 161–170 (1971).
- ⁵ Bombik, B. M., and Baserga, R., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2038–2042 (1974).
- ⁶ Gunther, G. R., Wang, J. L., and Edelman, G. M., *J. Cell Biol.*, **62**, 366–377 (1974).
- ⁷ Brooks, R. F., *J. cell. Physiol.*, **86**, 369–378 (1975).
- ⁸ Clarke, G. D., and Smith, C., *J. cell. Physiol.*, **81**, 125–132 (1973).
- ⁹ Schor, S., and Rozengurt, E., *J. cell. Physiol.*, **81**, 339–346 (1973).
- ¹⁰ Smith, J. A., and Martin, L., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1263–1267 (1973).
- ¹¹ Rubin, H., and Steiner, R., *J. cell. Physiol.*, **85**, 261–270 (1975).
- ¹² Smith, J. A., and Martin, L., in *Cell Cycle Controls*, (edit. by Padilla, G. M., Cameron, I. L., and Zimmerman, A.), 43–60 (Academic, New York, 1974).
- ¹³ Todaro, G. J., and Green, H., *J. Cell Biol.*, **17**, 299–313 (1963).
- ¹⁴ Vogt, M., and Dulbecco, R., *Proc. natn. Acad. Sci. U.S.A.*, **46**, 365–370 (1960).
- ¹⁵ Holley, R. W., and Kiernan, J. A., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 300–304 (1968).
- ¹⁶ Jainchill, J. L., and Todaro, G. J., *Expl Cell Res.*, **59**, 137–146 (1970).

T-lymphocyte response to Friend virus-induced tumour cell lines in mice of strains congenic at *H-2*

MICE immunised with infectious lymphocytic choriomeningitis virus (LCMV) produce T lymphocytes which can kill LCMV-infected target cells *in vitro* only if the killer and target cells are syngeneic at the *H-2K* and/or *H-2D* loci^{1,2}. We now report analogous results obtained in studies of the immune response of syngeneic or semi-syngeneic mice to cultured cell lines derived from tumours induced *in vivo* by Friend murine erythroleukaemia virus (FV). Our studies further indicate that the *H-2* type of the cells used for immunisation is also a decisive factor in the specificity of the killer cells generated.

The mice used were of strains congenic at the *H-2* region on the BALB/c (*H-2^d*) background: BALB.B

Table 1 T-killer cells in PEC from mice immunised with syngeneic or semi-syngeneic FV-induced tumour cells

Mice immunised*	Tumour cells used for immunisation and target cells	% ⁵¹ Cr release following various pretreatments of the PEC†			
		A	B	C	D
BALB.B	HFL/b	30	1	36	30
BALB.K	HFL/k	10	2		
(BALB.B × BALB.K)F ₁	HFL/b	20	4		
(BALB.B × BALB.K)F ₁	HFL/k	45	8		

In the LMC, 2.5×10^6 PEC in 1 ml and 5×10^4 ⁵¹Cr-labelled tumour cells in 0.1 ml (PEC–target cell ratio, 50 : 1) were mixed and incubated at 37 °C in 5% CO₂ atmosphere for 6 h. The cells were suspended in Medium 199 plus 20% foetal calf serum. Specific release of radioactivity into supernatant was compared with that from tumour cells alone frozen and thawed four times.

*PEC were collected at previously determined peaks of activity, 5 d after the second or third immunisation except for PEC obtained from the BALB.K anti-HFL/k immunisation which were collected after 7 d. Immunisation was done intraperitoneally with 5×10^6 tumour cells suspended in normal saline except in the case of BALB.K anti-HFL/k for which all immunisations after the first were with 10^7 cells.

†Pretreatments: A, normal mouse serum plus complement; B, mouse anti-Thy-1.2 plus complement; C, goat anti-mouse IgG plus complement; D, absorption on washed nylon column. 10^7 PEC were first incubated at 4 °C for 30 min in 0.4 ml of antiserum (1 : 2 dilution), then resuspended and incubated at 37 °C for 40 min in 0.6 ml of guinea pig serum (1 : 3 dilution) as a complement source.

(H-2^b from C57BL/10), BALB.K (H-2^k from C3Hf/An) and the F₁ cross from these two strains (BALB.B × BALB.K) F₁. The cultured tumour cell lines induced in mice of these strains were of the HFL series: HFL/b (BALB.B), HFL/k (BALB.K) and HFL/bk [(BALB.B × BALB.K)F₁] (ref. 3). All three cell lines express both the FV-induced FMR cell-surface antigen and the virus envelope antigens associated with the gp70 viral protein⁴. Each of the cell lines readily induces transplantation immunity in syngeneic hosts, with concomitant immunity to spleen focus induction by FV.

BALB.B, BALB.K and F₁ mice were immunised by intraperitoneal inoculation with H-2-compatible cultured tumour cells. Peritoneal exudate cells (PEC) from these mice, taken on day 5 or 7 after the second or third immunisation, were assayed for lymphocyte-mediated cytotoxicity (LMC) by the method of Berke *et al.*⁵. Targets for this assay were ⁵¹Cr-labelled cultured tumour cells or phytohaemagglutinin (PHA)-induced blasts originating from mice of the same congenic strains. The blasts were obtained from spleen cells incubated at 37 °C with PHA (1 : 100) for 3 d in an atmosphere of 5% CO₂.

Tables 1 and 2 show the results of our studies. LMC was completely abolished by pretreating the immune PEC with anti-Thy-1.2 and complement, whereas pretreating the immune PEC with normal mouse serum or goat anti-mouse IgG and complement or incubating the PEC at 37 °C for 45 min on a washed nylon column had no effect on ⁵¹Cr release (Table 1). Thus, target-cell killing in this system was mediated by T lymphocytes.

In agreement with other studies^{1,2,6}, we observed a requirement for shared H-2-region genes between the killer and target cells. PEC from BALB.B mice immunised with HFL/b tumour cells killed HFL/b and HFL/bk but not HFL/k target cells, and PEC from BALB.K mice immunised with HFL/k tumour cells killed HFL/k and HFL/bk but not HFL/b target cells. PHA blasts of either H-2^b or H-2^k type were not killed.

In previous studies of cellular immunity to virus-infected cells, the animals have been immunised directly with in-

fectious virus, which is, in effect, an immunisation with autochthonous infected cells^{1,2,6}. Since our immunisations were performed with cultured virus-induced tumour cells, we could study the response of F₁ mice immunised with parental strain tumour cells. From these experiments (Table 2) we have observed that T-cell killing also requires at least partial H-2 region identity on the part of the immunising cells and the tumour cells used as targets. Thus, PEC from (BALB.B × BALB.K)F₁ mice immunised with HFL/k killed only HFL/k and HFL/bk but not HFL/b target cells, although the immune PEC possessed an H-2 haplotype in common with all three target cells. Similarly, PEC from F₁ mice immunised with HFL/b killed HFL/b but not HFL/k target cells. These findings are analogous to those of Shearer *et al.* in which the T-killer cell response of F₁ mice to TNP-modified parental strain spleen cells was studied⁷.

A comparison of the responses of BALB.K and of F₁ mice to HFL/k cells suggests the involvement of an H-2-associated immune response gene. In BALB.K mice the peak response was reached on day 7 rather than day 5, required a larger immunising dose of tumour cells and produced a lower level of ⁵¹Cr release in tests on HFL/k target cells (Tables 1 and 2). It is conceivable that the presence of the H-2^b haplotype in the recipient confers a greater capacity for this immune response than the H-2^k haplotype.

To explain the requirement for common H-2 genes between T killer and target cells in virus-specific systems, two mechanisms have been proposed: (1) compatibility at H-2 is a physiological requirement for T-killer cell–tumour cell interactions specific for viral antigens; or (2) an alteration of the H-2 molecules on cell surfaces occurs as a result of virus infection, and these altered molecules, rather than viral antigens *per se*, are recognised as “non-self” by responding T-cell populations.

The first of these possible mechanisms implies that killer cells from (BALB.B × BALB.K)F₁ mice immunised with either HFL/k or HFL/b cells should kill both HFL/k and HFL/b cells since these killer cells share H-2 determinants with both parental strain tumour cell lines. Since F₁ T lymphocytes killed only the parental tumour cell with which they were immunised, this hypothesis does not seem correct.

If, according to the second proposed mechanism, H-2 molecules are altered as a result of the cell's infection by FV, two possible types of alteration may be envisaged: either the primary structure of the H-2 molecule itself is altered (pre-translational modification) or an interaction takes place between a viral molecule and an H-2 molecule (post-translational modification). In either case, the modification of H-2^b molecules must be of a similar nature on both HFL/b and HFL/bk cells, and the modification of H-2^k molecules must be similar on both HFL/k and HFL/bk cells although determinants on modified H-2^b and H-2^k molecules are not recognised as cross reacting

Table 2 Selective killing of ⁵¹Cr-labelled target cells by PEC from mice immunised with syngeneic or semi-syngeneic FV-induced tumour cells

Mice immunised	Tumour cells used for immunisation	% ⁵¹ Cr release from different target cells		
		HFL/b	HFL/k	HFL/bk
BALB.K	HFL/k	3	10	66
BALB.B	HFL/b	59	10	46
(BALB.B × BALB.K)F ₁	HFL/k	4	55	60
(BALB.B × BALB.K)F ₁	HFL/b	20	—5	

Methods as for Table 1.

antigens by the F_1 hosts. In spite of the possible occurrence of altered $H-2$ molecules on these cells, they continue to express high levels of unaltered $H-2$ specificities, as shown by their capacity to absorb antibodies to private $H-2$ specificities expressed on uninfected cells (unpublished data). Thus, if the alteration of the $H-2$ molecule is so extensive as to involve most of its antigenic features, then only a small proportion of $H-2$ molecules can be so altered; otherwise the alteration in $H-2$ must affect only a minor portion of the molecule.

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KENNETH J. BLANK
HERBERT A. FREEDMAN
FRANK LILLY

Department of Genetics,
Albert Einstein College of Medicine,
Bronx, New York 10461

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- 1 Zinkernagel, R. M., and Doherty, P. C., *Nature*, **248**, 701-702 (1974).
- 2 Doherty, P. C., and Zinkernagel, R. M., *J. exp. Med.*, **141**, 502-508 (1975).
- 3 Freedman, H. A., and Lilly, F., *J. exp. Med.*, **142**, 212-223 (1975).
- 4 Freedman, H. A., Lilly, F., and Steeves, R. A., *J. exp. Med.*, **142**, 1365-1376 (1975).
- 5 Berke, G., Sullivan, K. A., and Amos, B., *J. exp. Med.*, **135**, 1334-1348 (1972).
- 6 Chesebro, B., and Wehrly, K., *J. exp. Med.*, **143**, 85-99 (1976).
- 7 Shearer, G. M., Rehn, T. G., and Gorbarino, C. A., *J. exp. Med.*, **141**, 1348-1363 (1975).

Demonstration of sensory neurones in the ectopic cuticle of spineless-aristapedia, a homoeotic mutant of *Drosophila*

MANY mutations in *Drosophila melanogaster* cause the development of body parts in inappropriate places. Several of these homoeotic mutations (reviewed in ref. 1) result in the appearance of leg bristles and other leg cuticular structures in place of their antennal counterparts. In the mutant spineless-aristapedia (ss^a , 3-58.5) the antennal arista is replaced by tarsal segments²; the mutations Antennapedia ($Antp$, 3-48) and Nasobemia (Ns , 3-48) produce a more extreme transformation: most of the antennal cuticular structures are transformed into leg cuticle, and, in the latter, part of the head cuticle is replaced by sternopleural cuticle^{3,4}. In insects the sensory neurones are derived from epidermal cells⁵. When sensory epidermis is surgically transplanted to ectopic sites, neurones develop from it and form connections with the central nervous system⁶. These neurones act according to the type of epidermis from which they are derived rather than according to the position in which they develop⁶. In homoeotic mutants cuticular structures develop at ectopic sites as a result of genetic modification. In this report I examine whether in homoeotic mutants sensory neurones are formed, and, if so, whether they behave according to the homoeotic structures that develop, or according to their general position in the body.

Antennae of Canton-S (C-S) and ss^a flies were examined histologically by three methods. In the first, half-heads of C-S and ss^a flies were fixed in glutaraldehyde and embedded in Epon, and sections 1 μ m thick were cut and stained with methylene blue. In the second, flies' heads were fixed in Bouin's fluid, stained by the Bielchowsky silver technique, embedded in Epon and sectioned at 2.5 μ m. Finally, flies were frozen and sectioned at 8 μ m with a cryotome, and the sections were fixed and stained for acetylcholinesterase by the Karnovsky-Roots technique. The antennal tarsi of ss^a flies bear bristles, at the base of which can be seen nerve cells. By taking serial sections it was possible to show that

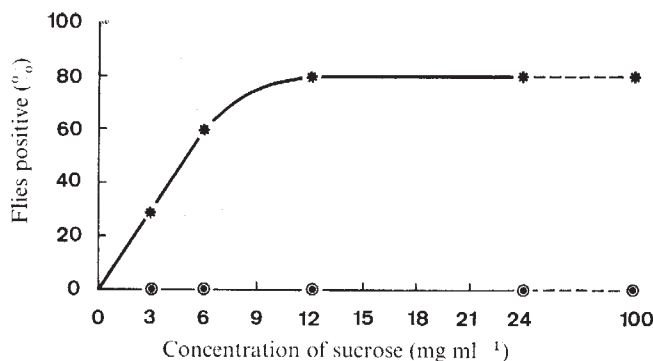


Fig. 1 Proportion of flies showing a proboscis-extension response when cuticular structures were stimulated with sucrose solutions of various concentrations. *, C-S legs; ○, C-S antennae. Each point is based on a minimum of 40 flies. The order in which the different appendages were treated with different solutions was varied randomly for each fly. For example, after being starved, attached to a slide, and water satiated (see text for details) a fly was tested as follows: five trials on tarsus of first left leg with sucrose (12.5 mg ml⁻¹), 10-min wait, during which the tarsus was washed, and the fly water satiated, five trials on left antennal tarsus with sucrose (6 mg ml⁻¹), 10-min wait, as above, five trials on tarsus of first right leg with sucrose (12.5 mg ml⁻¹) containing NaCl (150 mg ml⁻¹) and so on, for about 10 tests.

processes extend from these cells and join to form a small bundle of fibres, containing acetylcholinesterase, that passes proximally through the tarsal segments into the second antennal segment where it joins the antennal nerve, which runs into the brain. The first and second antennal segments of ss^a flies seem normal, and the Johnston's organ is indistinguishable from that in antennae of normal flies. No muscles were found in the ss^a tarsus.

Histological examination, therefore, shows that neurones develop from homoeotic cuticle. A simple behavioural test was used to see whether such neurones are sensory, and whether they behave like receptors in leg structures. When the tarsal chemoreceptors of a normal hungry fly are stimulated with sucrose solution, the fly extends its proboscis⁷. Stimulation of antennae with the same solution elicits no response. This behavioural test (the proboscis-extension response) can be used to establish the presence in the cuticle of gustatory chemoreceptors the stimulation of which results in proboscis extension.

Male and female flies (5-10 d old) of the different genotypes were kept for 18 h in vials without food but containing moist filter paper. They were then anaesthetised with carbon dioxide and attached, ventral side up, to a microscope slide, by a small drop of myristic acid (Baker) applied with a warm soldering iron. They were then left for 2 h to recover from the anaesthesia.

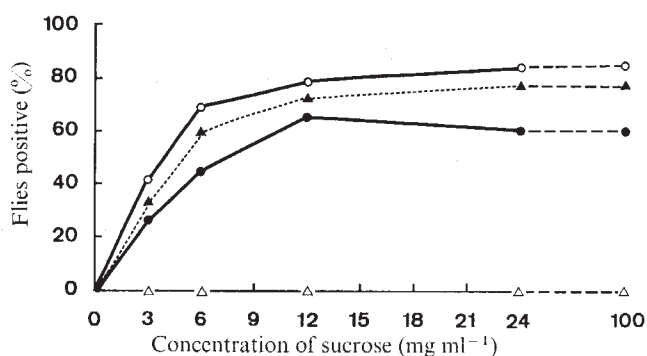


Fig. 2 Proportion of flies showing a proboscis-extension response when cuticular structures were stimulated with sucrose solutions of various concentrations. ○, ss^i legs; ●, ss^i arisal tarsi; ▲, $Antp^b$ legs; △, $Antp^b$ antennal legs. Each point is based on a minimum of 40 flies.

All flies were satiated with water before they were tested with sugar solutions. A droplet of water on the tip of an eyebrow hair was applied to one of the fly's tarsi. If the proboscis was extended in response, droplets were placed on the labellum and the fly was allowed to drink until further stimulation of the tarsus resulted in no extension of the proboscis—the fly then was water satiated. If this was not done the flies extended their probosces in response to water alone, which interfered with measurement of the dose-response curve for stimulation with sugar.

Presentation of a solution to a fly can modify its response to a second solution by changing the central excitatory state of the fly⁸. In C-S flies the response returns to normal within a few minutes (personal communication from Ghysen). To ensure that the central excitatory state was not influencing the flies' response, each was tested at 10-min intervals with solutions of different composition and concentrations, presented in random order. During each 10 min the test area of cuticle was washed with water to remove traces of the solution previously applied, and the fly was permitted to drink by placing a droplet of water on its labellum to ensure that it remained water satiated. Each test consisted of five consecutive applications at approximately

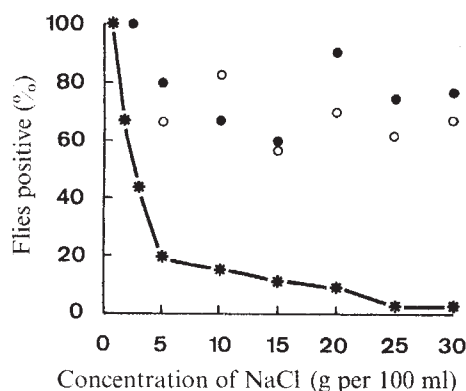


Fig. 3 Effect of NaCl on the response of tarsi to sucrose (12.5 mg ml^{-1}). *, C-S legs; O, ss^a legs; ●, ss^a arista tarsi. Only those flies showing a positive response to sucrose (12.5 mg ml^{-1}) were assayed; each point is based on a minimum of 20 flies.

20-s intervals of the test solution to one of the test areas (that is, one of the leg tarsi, one of the antennae, or one of the antennal legs). A test was scored as positive when it resulted in extension of the proboscis twice or more in the five trials.

The proboscis-extension response elicited when tarsi of the first legs of C-S flies were presented with sucrose solutions of various concentration is shown in Fig. 1. A maximum response (80%) was obtained with sucrose at a concentration of 12.5 mg ml^{-1} . Touching the antennae of these flies with the same solutions did not elicit a response (Fig. 1). About 20% of the flies did not respond to sugar at all; most of these were very inactive, and some died during the experiment.

Figure 2 shows that when the leg tarsi of ss^a flies were similarly stimulated the response was the same as that for C-S flies. When homoeotic antennal tarsi were stimulated a

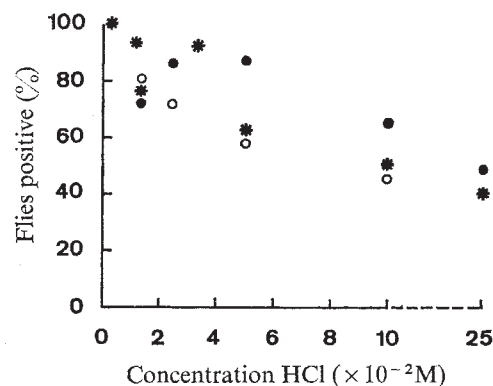


Fig. 4 Effect of HCl on the response of tarsi to sucrose (12.5 mg ml^{-1}). *, C-S legs; O, ss^a legs; ●, ss^a arista tarsi. Only those flies showing a positive response to sucrose were assayed; each point is based on a minimum of 20 flies.

similar but weaker response was obtained (Fig. 2). A smaller proportion (60%) of flies showed the response when their antennal tarsi were stimulated than when their leg tarsi were stimulated (80%), and those flies that responded did so less rapidly to stimulation of their homoeotic tarsi than of their leg tarsi. Most of the 20% of flies that did respond to leg tarsal stimulation were also not stimulated by application of sugars to their antennal tarsi. It is not clear why of the 80% of flies that did respond normally to stimulation of their leg tarsi one quarter were unresponsive when their homoeotic tarsi were stimulated.

To establish whether the tarsal chemoreceptors in the antennae of ss^a flies respond with the same specificity as those in the legs, several sugars were tested by the same procedure. Only those flies were tested that showed a proboscis-extension response when their tarsi were tested with 12.5 mg ml^{-1} sucrose. Table 1 shows that the specificity of response of the antennal tarsi in ss^a flies was the same for these sugars as that of leg tarsi in C-S and ss^a flies, that is, the homoeotic tarsus was responding to these sugars as if it were a normal leg tarsus.

Sodium chloride and other salts added to the sugar solution inhibit the proboscis-extension response⁹. Figure 3 shows the effect of sodium chloride on the response of flies to sucrose. The tarsi of C-S flies were sensitive to sodium chloride, while those of ss^a flies were relatively insensitive. This was true both for the normal and for the antennal tarsi of ss^a flies, showing that, although this mutant is abnormal with respect to its response to sodium chloride, the ss^a antennal tarsi still behave exactly like ss^a leg tarsi.

In *Phormia regina* hydrochloric acid added to the sugar solution also inhibits extension of the proboscis⁹. The same is partially true for *Drosophila melanogaster*; in the case of this inhibitor ss^a leg and antennal tarsi responded to the same extent as C-S tarsi (Fig. 4).

In the mutants *Antp*^h and *Ns* the antennae are largely transformed into leg structures^{3,4}. In both mutants the second and third antennal segments are partially or totally replaced by trochanteral, femoral and tibial structures¹⁰, while in *Ns* the first antennal segment includes more proximal leg structures, and the head bears sternopleural cuticle.

Table 1 Specificity of response of tarsi

Genotype	Structure tested	Melibiose	Sugar (100 mg ml^{-1})		
			Mannose	Fructose	Trehalose
C-S	Tarsus of first leg	19/53(36)	23/25(92)	25/25(100)	64/98(65)
	Antenna	0/50(0)	0/25(0)	0/25(0)	0/95(0)
ss^a	Tarsus of first leg	10/22(45)	19/19(100)	18/18(100)	21/42(50)
	Antennal tarsus	9/19(47)	14/15(93)	17/17(100)	19/40(47)

The figures given are the number of flies showing a positive response (that is, two or more proboscis extensions in five trials with the test sugar on the test area) out of the total number of flies tested with each sugar (percentages in parentheses).

Histological examination of femoral and tibial segments of the homoeotic legs showed that the bristles also have nerve cells at their bases. Axons from these cells run together through the leg and antennal segments to the antennal nerve, which passes into the brain. In *Antp^B* flies the Johnston's organ is markedly reduced in size; in *Ns* flies this is even more extreme. No muscles were found in the antennal legs of *Antp^B* flies; in *Ns* flies, muscles were only found in proximal homoeotic structures that correspond to the first antennal segment. In normal flies only the first antennal segment contains muscle; this perhaps explains why muscles are not found in the transformed second and third segments even though muscles would normally be present in the femur and tibia of the leg.

Stimulation of the antennal legs of *Antp^B* flies did not elicit extension of the proboscis, whereas stimulation of the tarsi of the legs produced a response (Fig. 2). The same was true for *Ns* flies. This is expected since in neither stock of these mutants were tarsi present on the antennal legs. Unlike that of *ss^a* flies, the sugar-elicited proboscis-extension response of the leg tarsi of *Antp^B* and *Ns* flies was inhibited by salt at the concentrations that inhibit the response of normal flies. Hydrochloric acid also had the same effect on these mutants as on normal flies.

These results show that in the homoeotic mutation *ss^a*, which results in ectopic development of tarsal cuticle, a sensory apparatus is produced that can transmit information which can be interpreted correctly and used by the central nervous system of the fly. This ectopic sensory structure acts according to the type of epidermis from which it is derived rather than according to the position in the body in which it develops. This finding confirms, by genetic methods, the results of surgical transplantation experiments such as those done with the cricket, in which transplantation of sensory structures to ectopic sites resulted in the formation of functional sensory neurones⁶.

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I. I. DEAK*

Division of Biology,
California Institute of Technology,
Pasadena, California 91125

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¹ Gehring, W. J., and Noethiger, R., in *Developmental Systems: Insects* (edit. by Counce, S. J., and Waddington, C. H.) (Academic, London, 1973).

² Balkaschina, E., *Arch. Entw. Mech. Org.*, **115**, 448–463 (1929).

³ Lewis, E. B., *Drosophila Inf. Serv.*, **30**, 76–77 (1956).

⁴ Gehring, W. J., *Arch. Julius Klaus Stift. Vererb. Forsch.*, **41**, 44–54 (1966).

⁵ Wigglesworth, V. B., *Q. J. microsc. Sci.*, **94**, 93–112 (1953).

⁶ Palka, J., and Schubiger, M., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 966–969 (1975).

⁷ Minnich, D. E., *Biol. Bull. mar. biol. Lab. Woods Hole*, **51**, 166–178 (1926).

⁸ Dethier, V. G., Solomon, R. I., and Turner, L. H., *J. comp. physiol. Psychol.*, **60**, 303–333 (1965).

⁹ Dethier, V. G., *Q. Rev. Biol.*, **30**, 348–371 (1955).

¹⁰ Postlethwait, J. H., and Schneiderman, H. A., *Devl Biol.*, **25**, 606–640 (1971).

EEG α asymmetry and visuospatial performance

THE discovery of complementary cognitive specialisation of the two cerebral hemispheres has led to speculation about its significance for understanding individual differences in cognitive style. It has been suggested^{1,2} that some people rely primarily on the left hemisphere whereas others rely on the right, and that this may be an important determinant of a person's cognition of his world.

Electroencephalographic (EEG) results have demonstrated that differential activation of the right or left hemisphere can be observed by analysing EEG α -band activity (8–12 Hz) during performance of appropriate cognitive activities. Since

α waves are usually taken to be an indicator of cortical deactivation³, relatively smaller α amplitudes in one hemisphere would presumably occur when that hemisphere is more active. A number of studies^{4–10} have found changes in the relative amounts of α activity in the two hemispheres which depend on the type of cognitive tasks a subject performs. The direction of this asymmetry is predictable from the known cognitive capabilities of each hemisphere. Linguistic tasks, such as composing a letter or mental arithmetic, show relatively more right deactivation. Similarly, visuospatial and musical tasks, which have been linked with right-hemisphere capabilities, show relatively more left deactivation.

If the suggestion that there are individual differences in hemispheric usage is correct, then it would be expected that persons who scored highest in objective tests of visuospatial abilities would have the lowest ratios of right-hemisphere α to left-hemisphere α . We tested this hypothesis by recording right and left posterior EEGs while subjects solved problems involving the imaginal manipulation of visually-presented forms. We have found that subjects with relatively less activity (and consequently more activation) in the right hemisphere perform more efficiently tasks which are known to rely on the right hemisphere.

Subjects were 16 right-handed students, recruited from an introductory course in psychology at Dartmouth College. The test of visuospatial imagery consisted of a series of 20 problems, selected from three different sources. Two types of problem were adapted from chronometric studies of visual imagery by Shepard and coworkers^{11,12}, and the third type was taken from a standardised test of spatial ability¹³. The three problem types were similar to each other in requiring the imaginary manipulation of objects in space, an ability shown to be preferentially impaired by right-posterior cortical lesions^{14,15} and performed poorly by the left hemisphere of commissurotomed patients^{16,17}. Figure 1 illustrates these problems.

The series of test items was presented to each subject in the same fixed order, in three blocks of trials separated by 2-min rest periods. Each problem was mounted on a card (5 × 8 inches) which the subject held in his hands during the test. Subjects were instructed to answer each problem verbally as soon as the answer was known, and to avoid double-checking their answers before responding. They were also instructed to keep their eyes open and to look at the card while solving the problem. Answers and time of response were recorded on the EEG polygraph protocols, and latencies were estimated to the nearest 0.5 s from the protocols.

Two EEG channels were recorded while the subject worked on the problems, and also during a 30-s eyes-open baseline before the problem series. The EEG channels were derived from homologous occipitoparietal sites: one at the midpoint between O_1 and P_3 ; the other halfway between O_2 and P_4 . Each of these was referenced to its ipsilateral earlobe. The EEGs were recorded conventionally with Grass gold-cup electrodes, Grass 7P511 amplifiers, and an Ampex PR500 tape recorder.

The recorded EEG signals were subsequently played back through matched α -bandpass filters (half-amplitude points at 8.5 and 12 Hz), and the output of the filters was integrated by circuits which reset every second. Two polygraph pens were driven by the integrators, and α amplitudes for each channel were measured by the distance the pens traversed on being reset. The output of this system was linear with the amplitude of a 10-Hz sine wave in the range of amplitude values used in this study.

Ratios of integrated α activity from the right hemisphere to α activity in the left hemisphere (R/L ratios) were computed for each 1-s epoch. A rank order plot of overall average ratios against average time to problem solution for each subject is shown in Fig. 2, indicating that those subjects with the lower R/L ratios solved the problems most efficiently. This result is in the direction predicted by the hypothesis of Bogen *et al.*¹ and is consistent with previous studies of EEG asymmetry^{4–10}. Subjects who have relatively less right- α activity and therefore

relatively more right activation, perform better on a putative right hemisphere task.

The correlation found here is nearly as strong if R/L ratios recorded during the resting baseline period are correlated with the average problem-solution latencies ($\rho = 0.508$, $P < 0.05$). Apparently, most of the correlation observed in these data is a product of a tonic asymmetry in the EEG rather than a momentary activation pattern.

Dumas and Morgan⁶ failed to find a relationship of the type reported here. These investigators used occupational category as the criterion variable for cognitive ability, arguing that a group of engineers would be expected to exhibit higher R/L α ratios than a group of artists. One difficulty with this research strategy is that there may be too many variables other than cognitive predisposition which determine occupational choice. Correspondingly, there may be, within any one occupation, great variety in the ways in which one can approach a task. For example, it is frequently observed that some mathematicians are clearly geometers—that is, they think about mathematics in visuospatial terms, and their proofs tend to be based on geometrical conceptions. There are also other mathematicians who are said to be algebraists, who tend to think mathematically

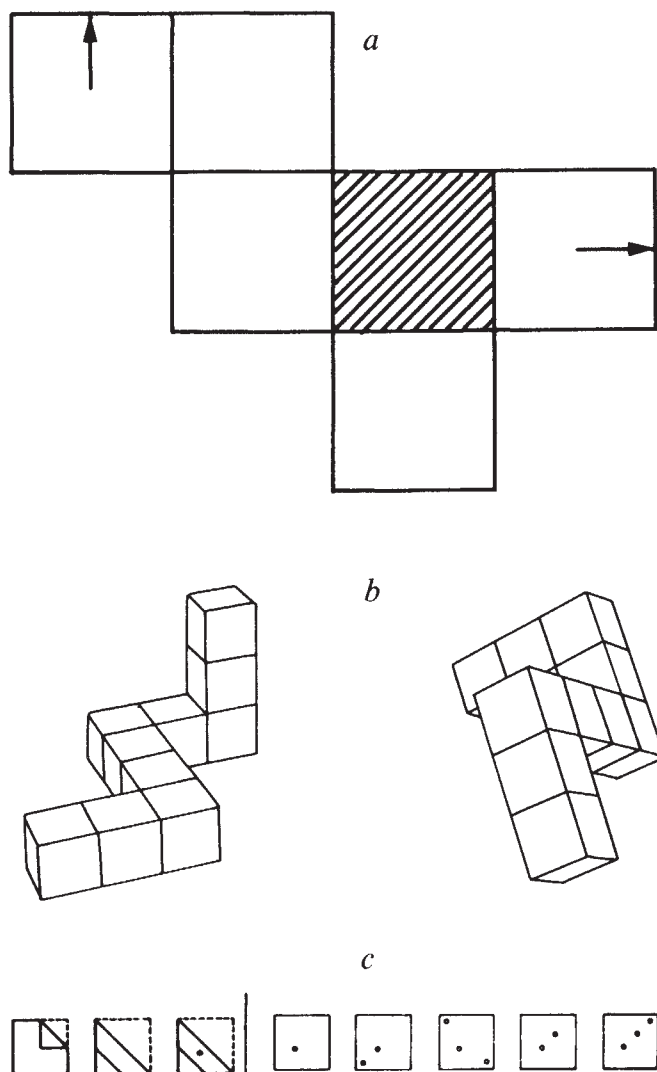


Fig. 1 Examples of the three types of visuospatial problems. *a*, Cube folding: mentally fold the figure into a cube, using the striped side as the base, and decide whether or not the two arrows meet (after ref. 12). *b*, Three-dimensional rotation: rotate in space one of the two figures and decide whether they are congruent or mirror opposites (after ref. 11). *c*, Paper folding: observe the sequence of folding operations in the left figures and decide the outcome of punching the indicated hole by choosing the correct figure on the right (from ref. 13).

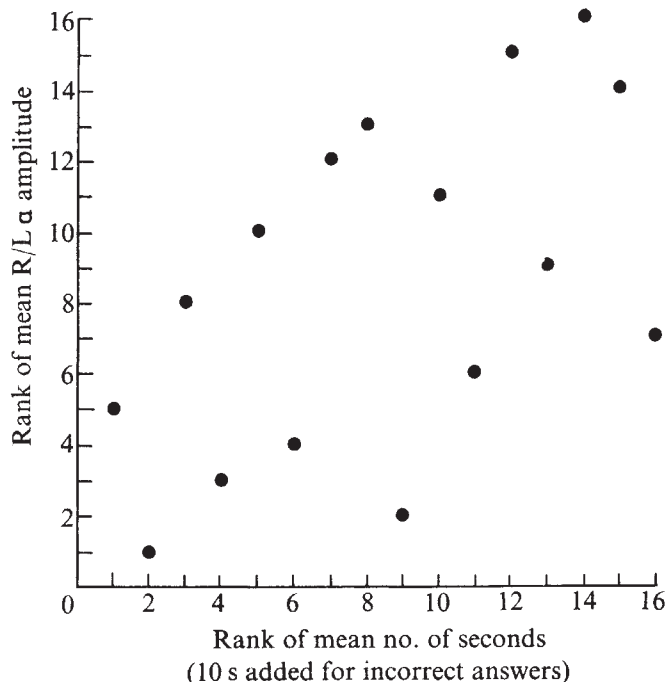


Fig. 2 Rank order scatter plot of average EEG asymmetry against average problem-solution latency. Ratios of right-to-left- α activity were computed for each 1-s epoch and averaged over each trial for each subject. Values shown here are subject averages over all problems. Rank data are given because of the skewness of the distribution of average latencies. These data produce a rank order correlation of 0.546 ($t = 2.44$, d.f. = 14, $P < 0.05$). The latencies ranked here have been corrected for errors by adding 10 s to the time to solution on incorrect trials. If no correction is made for incorrect answers, the value of the correlation is attenuated only slightly ($\rho = 0.515$, $P < 0.05$).

in terms of the propositional manipulation of symbols. Similar kinds of differences are likely to be found within any occupational category.

The subjects tested in the present investigation, being sampled from a group of bright college students, were no doubt selected in part for visuospatial ability. It is a well known statistical fact that such a restriction in the range of one variable tends to reduce the value of the correlation coefficient¹⁸, indicating that the present result might underestimate the correlation in the larger population.

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CHARLES J. FURST

Dartmouth College
Hanover, New Hampshire, 03755

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- Bogen, J. E., DeZure, R., TenHouten, W. D., and Marsh, J. F., *Bull. L. A. neur. Soc.*, **37**, 49–61 (1972).
- Galin, D., and Ornstein, R., *Neuropsychologia*, **12**, 367–376 (1974).
- Morrell, F., in *The Neurosciences* (edit. by Quarton, G. C., Melnechuk, T. M., and Schmitt, F. O.) (MIT, Cambridge, Massachusetts 1967).
- Morgan, A. H., McDonald, P. J., and MacDonald, H., *Neuropsychologia*, **9**, 459–469 (1971).
- Morgan, A. H., McDonald, H., and Hilgard, E. R., *Psychophysiology*, **11**, 275–282 (1974).
- Dumas, R., and Morgan, A., *Neuropsychologia*, **13**, 219–228 (1975).
- Galin, D., and Ornstein, R., *Psychophysiology*, **9**, 412–418 (1972).
- Doyle, J. C., Ornstein, R., and Galin, D., *Psychophysiology*, **11**, 567–578 (1974).
- McKee, G., Humphrey, B., and McAdam, D. W., *Psychophysiology*, **10**, 441–443 (1973).
- Robbins, K. I., and McAdam, D. W., *Brain Lang.*, **1**, 189–193 (1974).
- Shepard, R. N., and Metzler, J., *Science*, **171**, 701–703 (1971).
- Shepard, R. N., and Feng, C., *Cog. Psychol.*, **3**, 228–243 (1972).
- French, J. W., Ekstrom, R. B., and Price, L. A., *Kit of Reference Tests for Cognitive Factors* (Educational Testing Service, 1962).
- Hecan, H., in *Interhemispheric Relations and Cerebral Dominance* (edit. by Mountcastle, V. B.) (Johns Hopkins Press, Baltimore, 1962).
- Bogen, J. E., *Bull. L. A. neur. Soc.*, **34**, 135–162 (1969).
- Levy-Agresti, J., and Sperry, R. W., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 1151 (1968).
- Milner, B., and Taylor, L., *Neuropsychologia*, **10**, 1–15 (1972).
- McNemar, Q., *Psychological Statistics*, third ed. (Wiley, New York, 1962).

Bicuculline reversal of deprivation amblyopia in the cat

CATS deprived of visual experience in one eye during a critical period of early life develop marked changes in the functional organisation of their visual system¹⁻³. Useful vision seems to be lost in the deprived eye, and neurophysiological studies reveal a loss of binocular input to neurones in the visual cortex. Most neurones fail to respond to stimuli presented to the deprived eye; whereas almost all respond to stimulation of the normal eye. In normal neonatal kittens without visual experience, most cortical neurones respond to stimulation of either eye. Moreover, this binocular neuronal input persists when subsequent visual experience is prevented by binocular lid suture. Thus, asymmetrical visual experience seems necessary to produce a loss of binocular input. This loss of functional input from the deprived eye could result from a loss of the normal excitatory synaptic connections or from inhibitory suppression of afferent input. We postulate that asymmetrical visual experience causes synaptic inhibition of input from the deprived eye and that reduction of such inhibition might restore binocularity.

Several lines of evidence suggest that inhibition in the cat visual system may be mediated by γ -aminobutyric acid (GABA). Of particular importance are the reports that bicuculline, a GABA-receptor blocker⁴, is able to alter receptive field (RF) properties of normal visual neurones⁵⁻⁸. We, therefore, attempted to restore binocularity in monocularly deprived cats by intravenous administration of bicuculline. This drug was able to restore binocular input to more than half the cortical neurones tested.

Five kittens were monocularly deprived of vision by lid suture in their fourth week of life. After 8 months of monocular deprivation, extracellular single unit recordings were made in the area centralis of visual cortex. We used

Table 1 Response of neurones to bicuculline by RF type (responsive cells/total cells tested)

Cat	All cells	Simple	Complex	Hyper-complex
1	1/1	1/1	0/0	0/0
2	4/8	3/4	2/4	0/0
3	6/10	1/1	3/7	2/2
4	5/10	2/3	2/5	1/1
5	4/4	0/0	3/3	1/1
All:	20/33	7/9	10/19	4/4
	(60.61%)	(77.78%)	(52.63%)	(100%)

paralysed (gallamine triethiodide) and locally anaesthetised (xylocaine) preparations to avoid possible confounding of our results by the known neurophysiological effects of general anaesthesia⁹. Single-cell activity, electroencephalogram (EEG), stimulus marker, and a voice channel were tape recorded. Each eye was independently stimulated with moving slits and bars of light projected on a tangent screen on which the eye was focused by a contact lens. An insulated tungsten microelectrode was advanced while stimulating the normal eye. RFs of responsive cells were characterised as "simple, complex, or hypercomplex"^{10,11}. Next, the amblyopic eye was stimulated before and after the administration of intravenous bicuculline, usually given in 0.1–0.2-mg aliquots (0.03–0.06 mg kg⁻¹).

All 33 cells evaluated responded to stimulation of the normal eye and had normal RF patterns. In contrast, only one could be driven by the amblyopic eye. Few unresponsive cells were encountered. These results agree with other neurophysiological studies of monocular deprivation amblyopia¹⁻³.

Administration of intravenous bicuculline produced a striking change in more than half the cells studied. Nineteen cells now responded to stimulation of the amblyopic eye (Figs 1 and 2). Although the brief duration (Fig. 2) of the response limited our study, the new amblyopic receptive field of a given neurone seemed similar, if not identical, with respect to location, size, shape, orientation specificity and directional specificity to the RF found with stimulation of the normal eye. Cells of each RF type (simple, complex, hypercomplex) showed a response to bicuculline (Table 1). The amblyopic RF tended to show wider orientation tuning¹² than the pre-drug RFs to stimulation of the normal eye; however, most normal eye RFs showed a similar widening of their orientation tuning after bicuculline. The one cell responsive to stimulation of the deprived eye before bicuculline, which had shown an abnormal RF type, developed a more normal RF after bicuculline. Thus, relatively normal RFs to stimulation of the deprived eye were brought out in 20 of the 33 cells studied (60%). The remaining 13 cells (40%) failed to respond to stimulation of the amblyopic eye in spite of sufficient bicuculline to produce seizure discharges on EEG. The responses of 17 cells began within 30 s of bicuculline administration and lasted up to 10 min. The remaining 3 cells had a delayed response onset and a longer duration, lasting up to 30 min.

The administration of bicuculline was often complicated by its potent convulsive effects. Seizure discharges lasting 2–3 min often followed the bicuculline response within 15–30 s. We do not feel, however, that the restoration of binocularity was due to some nonspecific increase in excitability associated with seizure activity. All neurones which responded to stimulation of the deprived eye did so at subconvulsive doses of bicuculline. Moreover, the appearance of seizure discharges at higher dose levels was always associated with loss of any response from the amblyopic eye. Finally, in two cells which had previously responded to bicuculline, we found that intravenous physostigmine failed

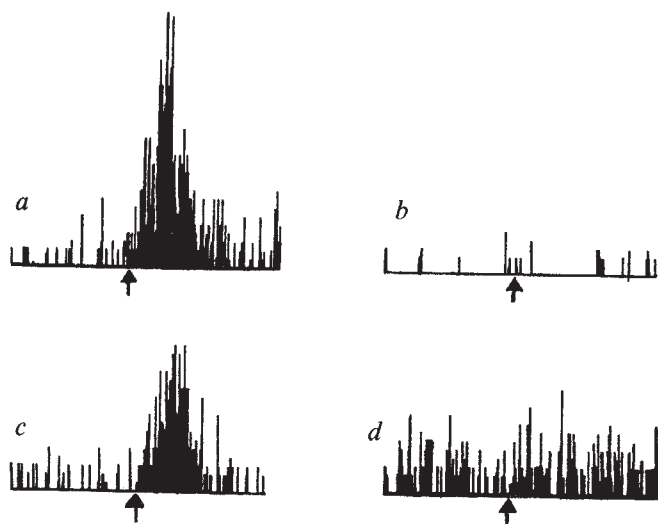


Fig. 1 Stimulus-related response histograms for monocular stimulation ($n = 10$) of a single cell in cat visual cortex with a complex RF. Time base is 512 ms. Arrow indicates entry of light-bar stimulus into the receptive field of the cell. Same stimulus parameters (size, shape, orientation, direction and speed) used for each histogram. *a*, Normal eye open, pre-drug; *b*, amblyopic eye open, pre-drug: no response; *c*, amblyopic eye open, 2 min after 0.06 mg kg⁻¹ of intravenous bicuculline: note response to stimulation and its similarity to *a*; *d*, amblyopic eye open, 15 min after bicuculline: note disappearance of response in spite of higher background firing rate. 20 of 33 cells developed similar responsiveness to stimulation of amblyopic eye after bicuculline which was independent of changes in background firing rate.

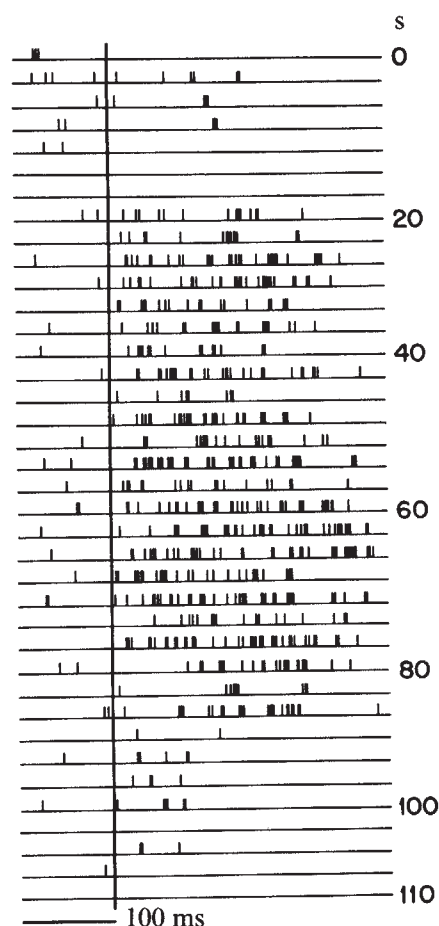


Fig. 2 Neuronal activity (Schmitt trigger output) of a complex visual cortical cell during monocular stimulation of the deprived eye. The continuous vertical line indicates entry of an optimally oriented light bar into the RF. Time base is 400 ms. Responses of the cell to sequential stimuli are shown in temporal order from top to bottom. The first line is immediately after 0.03 mg kg⁻¹ of intravenous bicuculline. Time (s) after bicuculline is shown to the right. Note the rapid onset of response at 20 s and its disappearance by 90–100 s. At higher dose levels, binocularity could be restored for longer periods of time.

to restore binocularity even at dose levels producing seizure activity.

Thus, our results indicate that intravenous bicuculline can make visual cortical neurones accessible to stimulation of the deprived eye in cats with monocular deprivation amblyopia. Access from the deprived eye could be restored after full development of the amblyopic condition with 8 months of visual deprivation. Moreover, the amblyopic eye RF showed the same properties as the normal eye RF. These observations suggest that the pathways between the amblyopic eye and the visual cortex may be capable of normal physiological function in spite of morphological changes which have been found in such preparations¹³. Since bicuculline was given intravenously, our data do not indicate where it acts to restore binocularity.

We postulate that the restoration of binocular input by bicuculline occurs by reduction of inhibitory mechanisms by way of GABA-receptor blockade. This mechanism is compatible with available information about the cat visual system. First, inhibitory input has been proposed as an important determinant of the normal response characteristics of visual neurones^{14,15}. Second, GABA has been implicated as a visual inhibitory transmitter^{16,17}. Finally, bicuculline alters the RFs of normal visual neurones in a manner consistent with reduction of inhibition³⁻⁸. If our interpretation is correct, then the total dominance by the normal eye results from active inhibition of the relatively

intact input from the amblyopic eye and suggests that feline amblyopia may not involve a permanent, irreversible loss of visual function.

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FRANK H. DUFFY

Seizure Unit Neurophysiology Laboratory,

S. ROBERT SNODGRASS

Neurology Research Laboratory,

JAMES L. BURCHFIELD

JANET L. CONWAY

Seizure Unit Neurophysiology Laboratory,

Department of Neurology,

Harvard Medical School,

Children's Hospital Medical Center,

Boston, Massachusetts 02115

Received November 14, 1975; accepted February 6, 1976.

- ¹ Wiesel, T. N., and Hubel, D. H., *J. Neurophysiol.*, **28**, 1029–1040 (1965).
- ² Wiesel, T. N., and Hubel, D. H., *J. Neurophysiol.*, **28**, 1060–1072 (1965).
- ³ Barlow, H. B., *Nature*, **258**, 199–204 (1975).
- ⁴ Curtis, D. R., and Johnson, G. A. R., *Ergebn. Physiol.*, **69**, 98–188 (1974).
- ⁵ Rose, D., and Blakemore, C., *Nature*, **249**, 375–377 (1974).
- ⁶ Daniels, J. D., and Pettigrew, J. D., *Brain Res.*, **93**, 41–62 (1975).
- ⁷ Sillito, A. M., *J. Physiol., Lond.*, **250**, 287–304 (1975).
- ⁸ Sillito, A. M., *J. Physiol., Lond.*, **250**, 305–329 (1975).
- ⁹ Ikeda, H., and Wright, M. J., *Expl Brain Res.*, **20**, 471–484 (1974).
- ¹⁰ Hubel, D. H., and Wiesel, T. N., *J. Physiol., Lond.*, **160**, 106–154 (1962).
- ¹¹ Hubel, D. H., and Wiesel, T. N., *J. Neurophysiol.*, **28**, 229–289 (1965).
- ¹² Rose, D., and Blakemore, C., *Expl Brain Res.*, **20**, 1–17 (1974).
- ¹³ Chow, K. L., in *Handbook of Sensory Physiology: Central Visual Information*, A, 7/3 (edit. by Jung, R.), 599–627 (Springer, Berlin, Heidelberg, New York, 1973).
- ¹⁴ Creutzfeldt, O., and Ito, M., *Expl Brain Res.*, **6**, 324–352 (1968).
- ¹⁵ Benevento, L. A., Creutzfeldt, O. D., and Kuhnt, U., *Nature new Biol.*, **238**, 124–126 (1972).
- ¹⁶ Iversen, L. L., Mitchell, J. F., and Srinivasan, V., *J. Physiol., Lond.*, **212**, 519–534 (1971).
- ¹⁷ Wallingford, E., Ost Dahl, R., Zarzecki, P., Kaufman, P., and Somjen, G., *Nature new Biol.*, **242**, 210–212 (1973).

Dopamine release in substantia nigra?

IN addition to the axons which project to the nucleus caudatus^{1,2}, the dopamine (DA)-containing perikarya of the substantia nigra have extensive dendritic processes. These dendrites, observed after Golgi staining³⁻⁵ and by electron microscopy^{3,4}, contain relatively large amounts of DA—revealed by histofluorescence⁶—and vesicle-like structures⁴. The DA seems to be stored in reserpine-sensitive structures⁶. The question has arisen whether DA can be released from the dendritic processes, as well as from nerve terminals of the nucleus caudatus^{7,8}. Our investigation of the metabolism of released DA suggests that it can.

3,4-Dihydroxyphenylacetic acid (DOPAC) is the main metabolite of striatal and limbic DA and less than 50% of DOPAC is converted to homovanillic acid (HVA)⁹. We have investigated the metabolism of DA after electrically stimulated release in areas containing exclusively nerve terminals (nucleus caudatus¹⁰, nucleus accumbens and the olfactory tubercle) and in a mesencephalic area devoid of nerve endings, but containing cell bodies (groups A₈, A₉ and A₁₀)^{11,12} and DA-rich dendrites⁸. Rats (males, 200–230 g, Wistar-derived strain) were anaesthetised with chloral hydrate (400 mg kg⁻¹, intraperitoneally) and placed in a stereotaxic device. A monopolar electrode was inserted through a burr hole in the exposed skull, with the coord-

Table 1 Alterations of striatal concentrations of DA, DOPAC and HVA after electrical stimulation of MFB

Compound	Concentration in corpus striatum ($\mu\text{g g}^{-1} \pm \text{s.e.m.}$)		
	Ipsilateral	Contralateral	Difference
DA (7)	9.05 ± 0.40	10.74 ± 0.28	1.70 ± 0.24
HVA (6)	2.54 ± 0.16	1.77 ± 0.09	0.77 ± 0.15
DOPAC (9)	3.30 ± 0.16	1.78 ± 0.11	1.52 ± 0.24

Stimulation was for 10 min, 30 Hz, 300 μA . Number of experiments in parentheses. All differences between ipsilateral and contralateral sides $P < 0.005$.

Table 2 Alterations of the concentration of DOPAC in various areas in the brain after stimulation of MFB

Brain area (N)	Concentration of DOPAC $\pm$ s.e.m. (μ g wet weight)		
	Ipsilateral	Contralateral	Difference
Antidromic activation			
Mesencephalic area including cell bodies + dendrites (11)	0.39 $\pm$ 0.02	0.26 $\pm$ 0.01	0.13 $\pm$ 0.02
Orthodromic activation			
Olfactory tubercle (10)	3.35 $\pm$ 0.14	1.83 $\pm$ 0.11	1.51 $\pm$ 0.14
Nucleus accumbens (6)	3.31 $\pm$ 0.21	2.27 $\pm$ 0.19	1.04 $\pm$ 0.20
Corpus striatum (9)	3.30 $\pm$ 0.16	1.78 $\pm$ 0.11	1.52 $\pm$ 0.24

N, Number of observations (in parentheses). All differences between ipsi- and contralateral sides are significant at $P < 0.005$. Stimulus parameters: 300 μ A; biphasic; frequency 30 Hz; duration 10 min.

inates as described before⁸. The tip of the electrode was placed in the median forebrain bundle (MFB) which was stimulated electrically for 10 min (biphasic constant current of 300 μ A; frequency 30 Hz; pulse length 2 ms). These stimulation parameters were found to give a maximal release of DA, and subsequent formation of DOPAC and HVA^{8,12}. After stimulation we dissected various brain areas, including the nucleus caudatus, nucleus accumbens, olfactory tubercle and 12 mg of mesencephalic tissue containing both the substantia nigra (catecholamine groups A₈ and A₉) and group A₁₀ of the ventral tegmental area, as described before^{8,9,13,14}.

In all cases the concentrations of the metabolites of DA in areas ipsilateral to the electrode were compared with those in the corresponding contralateral areas. DOPAC, HVA and DA were analysed as before^{8,13,14}.

Within 10 min of stimulation striatal DA was decreased by about 20%, while the concentration of DOPAC and HVA had increased (Table 1). The rise of the concentration of DOPAC was about double that of HVA, indicating that DA after release is predominantly deaminated. In these stimulatory conditions the potent inhibitor of DA uptake, nomifensine, had no effects on the metabolism of DA, demonstrating that there is no reuptake of released amine⁶. In all areas studied, DOPAC was increased by electrical stimulation of the MFB, thus including the non-terminal areas of the brainstem, which are antidromically activated (Table 2). These observations strongly suggest that when a nerve impulse is generated in dopaminergic neurones, DA is released not only from nerve terminals, but also from the cell bodies and dendrites. In addition we found (unpublished) that various drugs, including morphine, oxotremorine and neuroleptics, increase the concentrations of DOPAC and HVA not only in striatal and limbic structures, but also in the mesencephalic area containing dopaminergic cell bodies and dendrites.

What function can be attributed to DA released from non-terminal structures? We shall consider three possibilities. First, DA may inhibit the neurone from which it is released (self- or auto-inhibition). Bunney and Aghajanian¹⁵ found that DA-containing neurones of the substantia nigra were inhibited when the amine was applied microiontophoretically in the vicinity on the perikarya. With this proposed mechanism dopaminergic neurones may restrict the range of their firing frequencies. Second, DA released in the substantia nigra may inhibit other neurones, located near the dendrites releasing the amine (lateral inhibition). Such mechanisms have been described in other areas in the central nervous system, including the retina. Dendro-dendritic contacts in the substantia nigra have been revealed by electron microscopy⁴. Groves *et al.*¹⁶ found that local infusion of amphetamine, a drug which causes release of DA, also inhibits neuronal activity in the substantia nigra. This finding, however, does not discriminate between the two hypotheses.

The third possibility is that dopaminergic neurones have no preferential orientation. Thus the observed dendrites can be

considered to have the same function as the aminergic varicosities observed in the other parts of the brain. Neurones without obvious polarity include the retinal amacrine cells, of which a subpopulation seems to use DA as a neurotransmitter¹⁷. Which of these possibilities, or combination of possibilities, is valid for dopaminergic cells of the substantia nigra cannot be decided from available knowledge, including the data reported here. Our data do show that released DA is rapidly converted to DOPAC, not only in areas of the brain containing nerve terminals, but also where only perikarya and dendrites are found. These results challenge the concept that DOPAC is formed exclusively intraneuronally and that release of a neurotransmitter occurs only from nerve terminals.

JAKOB KORF

MARRIE ZIELEMAN

Department of Biological Psychiatry

BEN H. C. WESTERINK

Pharmaceutical Laboratories,
Department of Clinical Chemistry,
Groningen University,
Oostersingel 59, Groningen,
The Netherlands

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- 1 Ungerstedt, U., *Acta physiol. scand.*, Suppl., **367**, 1-48 (1971).
- 2 Lindvall, O., and Björklund, A., *Acta physiol. scand.*, Suppl., **412**, 1-48 (1974).
- 3 Rinvik, E., and Grofova, I., *Expl Brain Res.*, **11**, 229-248 (1970).
- 4 Hajdu, F., Hassler, R., and Bak, I. J., *Z. Zellforschung*, **146**, 207-221 (1973).
- 5 Cajal, S., Ramon, Y., *Histologie du Système Nerveux de l'Homme et des Vertébrés*, **2**, 275-278 (Cousejo Superior de Investigaciones Científicas, Inst. Ramon y Cajal, Madrid, 1955).
- 6 Björklund, A., and Lindvall, O., *Brain Res.*, **83**, 531-537 (1975).
- 7 McLennan, H., *J. Physiol., Lond.*, **174**, 152-161 (1964).
- 8 Korf, J., Grasdijk, L., and Westerink, B. H. C., *J. Neurochem.* (in the press).
- 9 Westerink, B. H. C., and Korf, J., *Eur. J. Pharmac.* (in the press).
- 10 Roth, R. H., Walter, J. R., and Aghajanian, G. K., *Biochem. Pharmac.*, Suppl., **457-464** (1974).
- 11 Dahlström, A., and Fuxe, K., *Acta physiol. scand.*, **62**, Suppl., 232, 1-55 (1964).
- 12 Von Voigtlander, P. F., and Moore, K. E., *Neuropharmacology*, **10**, 733-741 (1971).
- 13 Westerink, B. H. C., and Korf, J., *Eur. J. Pharmac.*, **33**, 31-40 (1975).
- 14 Westerink, B. H. C., and Korf, J., *Eur. J. Pharmac.* (in the press).
- 15 Bunney, B. S., and Aghajanian, G. K., *Biochem. Pharmac.*, Suppl., **792-797** (1974).
- 16 Groves, P. M., Wilson, C. J., Young, S. J., and Rebec, G. V., *Science*, **190**, 522-529 (1975).
- 17 Stell, W. K., in *Handbook of Sensory Physiology*, **7**, Part 2, *Physiology of Photoreceptor Organs* (edit. by Fuortes, M. G. F.), 111-213 (Springer, Berlin, Heidelberg, New York, 1972).

Release of dopamine from dendrites in rat substantia nigra

THERE is increasing evidence that neurotransmitter synthesis, storage and release are not confined to axon terminals. Much of this evidence has come from studies of the dopaminergic neurones of the substantia nigra (see Fig. 1). The terminal dendritic processes of dopamine (DA) neurones in substantia nigra are varicose and contain clusters of small vesicles^{1,2}; histochemical studies have shown that the dendrites contain DA and are able to take it up^{3,4}. There is also evidence that the components necessary for the synthesis and storage of DA are transported

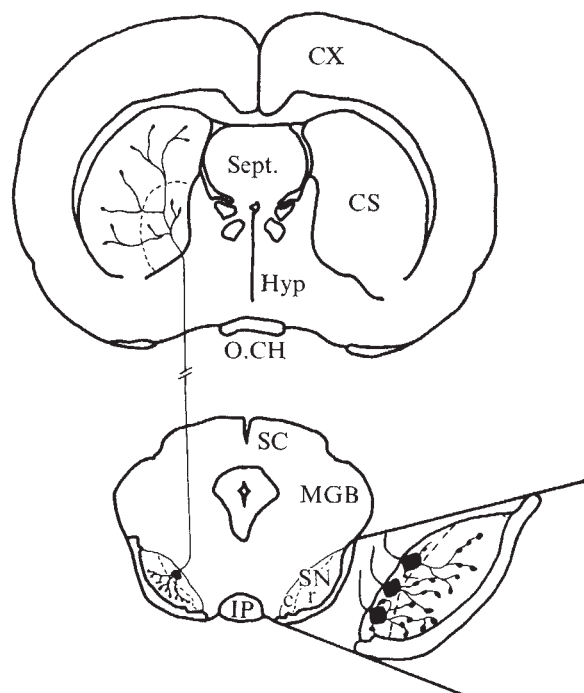


Fig. 1 Schematic representation of the dopaminergic nigro-striatal pathway. The cell bodies of dopaminergic neurones are largely confined to the pars compacta of substantia nigra with a well defined anatomical segregation of their axonal and dendritic processes¹. Within substantia nigra, the dendrites of these neurones radiate ventrolaterally into the pars reticulata, whereas the axons project rostromedially ending mainly in the corpus striatum. Some dendrites are also present in the pars compacta. CX, Cerebral cortex; CS, corpus striatum; Sept., septum pellucidum; Hyp., hypothalamus; O.Ch, optic chiasma; SC, superior colliculi; MGB, medial geniculate body; IP, nucleus interpeduncularis; SN, substantia nigra; c, pars compacta of the substantia nigra; r, pars reticulata of the substantia nigra.

somatofugally in dendrites of substantia nigra^{3,5}. Finally, the local application of DA agonists to the substantia nigra produces an inhibition of firing of neurones in the pars compacta that is blocked by DA antagonists^{6,7}. On the basis of these observations, it has been proposed that a local dendritic release of DA may modify the excitability of dopaminergic neurones in substantia nigra⁷. There has, however, been no direct demonstration of transmitter release from these or other dendrites. In this study we present evidence for the release of DA from dendrites of dopaminergic neurones in rat substantia nigra.

We have used a combination of methods that provides a refinement of previous techniques for the study of the release of neurotransmitters in the central nervous system (CNS), enabling separate superfusion of fresh, microdissected areas of rat brain rich in cell bodies, dendrites or axon terminals of the same neurones.

The entire substantia nigra and samples of corpus

striatum were dissected from serial slices (300 μ m) of rat brain using the method of Zigmond and Ben Ari⁸. The pieces of tissue were incubated in ³H-DA and uptake of labelled amine was measured after a 15-min incubation (Table 1). The endogenous DA content of similar samples was measured by a radiochemical enzymatic method⁹. ³H-DA uptake per unit wet weight of tissue was 57% higher in corpus striatum than in substantia nigra, whereas the striatal DA concentration was more than sevenfold higher. Expressed in terms of endogenous DA concentration, therefore, ³H-DA uptake and retention was greater in substantia nigra than in corpus striatum. One explanation of this finding is that vesicles in dendrites may have a lower endogenous transmitter content and a higher capacity for retention of exogenous DA than vesicles in axon terminals. In sympathetic neurones, axonal vesicles have been shown to increase their noradrenaline content fourfold on migration down the axon¹⁰, with a concomitant decrease in capacity for uptake and retention of exogenous noradrenaline¹¹.

The evoked release of neurotransmitters from axon terminals in CNS by depolarising potassium concentrations has been extensively studied *in vitro*^{12,13}. Slices of substantia nigra and corpus striatum were labelled with ³H-DA and superfused with Krebs bicarbonate solution. The spontaneous release of ³H-DA was initially rapid but became stable after 20 min superfusion. Brief exposure to high potassium (23.5 mEq l⁻¹) evoked large and reproducible increases in ³H-DA efflux from both substantia nigra and corpus striatum (Fig. 2a and b). Slices of substantia nigra released a significantly higher proportion of their ³H-DA content than corpus striatum, both spontaneously and in response to potassium stimulation (Table 2). This may be related to the observation that the specific activity of ³H-DA in substantia nigra was considerably greater than in corpus striatum (Table 1).

The spontaneous efflux of ³H-DA from both tissues was unaffected by removal of calcium from the perfusion medium, but potassium-evoked release was reduced by 88 $\pm$ 2% in substantia nigra and 85 $\pm$ 6% in corpus striatum in the absence of calcium (mean $\pm$ s.e.m. $n=4$) (Fig. 2c and d). In each case, potassium-evoked release of ³H-DA could be restored by a brief exposure to normal calcium. Raising the magnesium concentration in the presence of calcium also reduced the potassium-evoked release, by 59 $\pm$ 1% in substantia nigra and by 72 $\pm$ 10% in corpus striatum (mean $\pm$ s.e.m., $n=4$) (Fig. 2c and d). There were no significant differences between substantia nigra and corpus striatum in the inhibitory effects of low calcium or high magnesium concentrations. Since the release of neurotransmitters by exocytosis is known to be calcium dependent and to be inhibited by high magnesium concentrations¹⁴, it is likely that DA released from corpus striatum and substantia nigra was vesicular in origin.

Further evidence for vesicular release was obtained by estimating the proportion of radioactive metabolites of

Table 1 Dopamine uptake and content in rat substantia nigra and corpus striatum

	Tissue wet weight (mg)	Endogenous DA (ng mg ⁻¹)	³ H-DA uptake (ng mg ⁻¹)	³ H-DA/endogenous DA ratio
Substantia nigra (cell bodies and dendrites)	3.11 $\pm$ 0.22 (12)	0.34 $\pm$ 0.02 (4)	1.22 $\pm$ 0.10 (7)	3.59
Corpus striatum (axon terminals)	6.16 $\pm$ 0.33 (10)	2.55 $\pm$ 0.31 (4)	1.92 $\pm$ 0.16 (7)	0.75

Adult male Wistar rats were anaesthetised and perfused through the left ventricle of the heart with 100 ml Krebs phosphate solution at 4 $^{\circ}$ C. Coronal slices (300 μ m thick) of the brain were cut in a 4 $^{\circ}$ C cold room with a McIlwain tissue chopper, and the entire substantia nigra and a portion of corpus striatum at the level of the optic chiasma were dissected using a stereomicroscope. Tissue samples were weighed and preincubated in Krebs-Henseleit solution at 37 $^{\circ}$ C for 5 min. ³H-DA (specific activity 8.95 Ci mmol⁻¹; NEN Chemicals) was added to a final concentration of 1 μ M and incubation continued for a further 15 min. After rinsing, the tissue radioactivity in acid methanol extracts of nigral and striatal tissue was shown by ascending chromatography (*n*-butanol-acetic acid-water, 4:1:1 v/v) to migrate with authentic DA. Each value is mean $\pm$ s.e.m. Number of samples in parentheses.

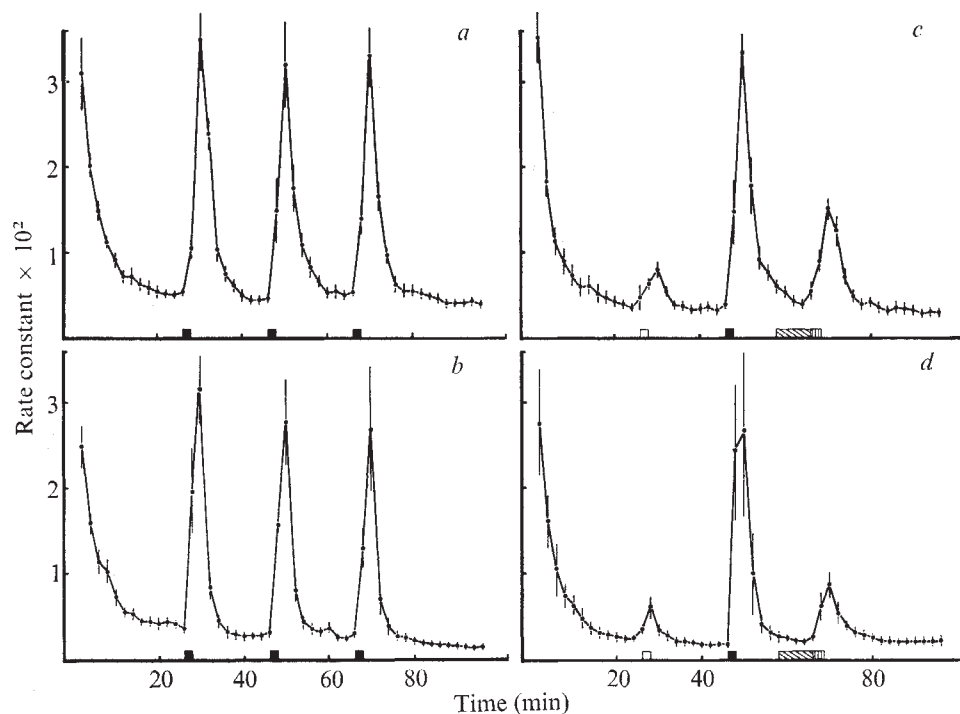


Fig. 2 Release of ^3H -DA from superfused slices of rat substantia nigra (a, c) and corpus striatum (b, d). Tissues were superfused at a rate of 30 ml h^{-1} with normal Krebs bicarbonate solution. At 20-min intervals, pulses of high potassium (23.5 mEq l^{-1}) were given for 2 min. In a and b, potassium pulses were given during perfusion with normal Krebs (■); in c and d, superfusion throughout the experiment was with calcium-free Krebs. The first potassium pulse was applied in the continued absence of calcium (□). For the duration of the second (■) and third (vertical hatching) potassium pulses the calcium concentration was restored to normal. Four fractions before, and during the third potassium pulse, the magnesium concentration was raised to 24 mEq l^{-1} (diagonal hatching); after the third potassium pulse, the magnesium concentration was restored to normal. Efflux of radioactivity was expressed as the fractional rate constant (d.p.m. released per min in superfusate/d.p.m. in tissue stores at time of collection). Each value is mean $\pm$ s.e.m. of four experiments.

^3H -DA in the superfusates. During stimulation of sympathetic nerves it has been shown that the ratio of released noradrenaline to its metabolites increases greatly¹⁵, presumably because transmitter released by exocytosis is not susceptible to intraneuronal metabolism. In some of the present experiments samples of superfusate were chromatographed on alumina columns¹⁶. The proportion of total radioactivity recovered in the catechol fraction was $60.1 \pm 4.5\%$ in basal samples and $90.5 \pm 1.7\%$ during the peak of the potassium-evoked release in both substantia nigra and corpus striatum (mean $\pm$ s.e.m., $n=4$).

To minimise the possible contribution to ^3H -DA release made by dopaminergic cell bodies in the substantia nigra, further experiments were carried out using only the pars reticulata of substantia nigra, removed by careful micro-

neurones or presynaptically on the axon terminals innervating them, or both. Release of transmitter close to cell bodies could also provide a mechanism for the local control of neuronal metabolism. A third regulatory function for the dendritic release of transmitter could be in the control of plastic and regenerative sprouting of neuronal processes and the establishment of new synapses.

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L. B. GEFFEN

Human Physiology Unit,
School of Medicine,
Flinders University of South Australia,
Bedford Park, Australia

T. M. JESSELL
A. C. CUELLO
L. L. IVERSEN

MRC Neurochemical Pharmacology Unit,
Department of Pharmacology,
Medical School, Hills Road,
Cambridge CB2 2QD, UK

Table 2 Fractional release rates of ^3H -DA from substantia nigra neurones

	Spontaneous	K ⁺ evoked
Substantia nigra (cell bodies and dendrites)	0.50 ± 0.02	5.76 ± 0.33
Corpus striatum (axon terminals)	$0.32 \pm 0.03^*$	$4.25 \pm 0.54^\dagger$

Values for spontaneous efflux are expressed as the mean $\pm$ s.e.m., ($n=12$) percentage of tissue ^3H -DA stores released in the three fractions before each potassium pulse; for potassium-evoked release as the total increase in ^3H -DA release over basal level for each pulse. Significance of difference between substantia nigra and corpus striatum: $^*P < 0.001$; $^\dagger P < 0.05$.

dissection. Each potassium pulse released $7.8 \pm 0.5\%$ (mean $\pm$ s.e.m., $n=4$) of ^3H -DA stores in pars reticulata. Thus, although it is not yet possible to assess the magnitude of ^3H -DA release from neurone cell bodies in the pars compacta it is clear that a substantial proportion of the ^3H -DA release observed in experiments on the entire substantia nigra occurs from dendritic processes.

The release of transmitter from dendrites may have a number of functions. Coordination of excitability of dopaminergic neurones within substantia nigra could be mediated by a DA receptor located either on the DA

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- Ramon y Cajal, S., *Estructura del sistema nervioso del hombre y de los vertebrados* (Madrid, 1904).
- Hajdu, F., Hassler, R., and Bak, I. J., *Z. Zellforsch.*, **146**, 207–221 (1973).
- Bjorklund, A., and Lindvall, O., *Brain Res.*, **83**, 531–537 (1975).
- Parizek, J., Hassler, R., and Bak, I. J., *Z. Zellforsch.*, **115**, 137–148 (1971).
- Pickel, V. M., Joh, T. H., Field, P. M., Becker, C. G., and Reis, D. J., *J. Histochem. Cytochem.*, **23**, 1–12 (1975).
- Aghajanian, G. K., and Bunney, B. S., *Frontiers in Catecholamine Research* (edit. by Usdin, E., and Snyder, S.), 643–648 (Pergamon, New York, 1973).
- Groves, P. M., Wilson, C. J., Young, S. J., and Rebec, G. V., *Science*, **190**, 522–529 (1975).
- Zigmond, R. E., and Ben-Ari, Y., *J. Neurochem.* (in the press).
- Cuello, A. C., Hiley, R., and Iversen, L. L., *J. Neurochem.*, **21**, 1337–1340 (1973).
- Lagercrantz, H., Kirksey, D. F., and Klein, R. L., *J. Neurochem.*, **23**, 769–774 (1974).
- Haggendal, J., and Dahlström, A., *J. Pharm. Pharmacol.*, **24**, 565–574, (1972).
- Mitchell, J. F., in *Methods in Brain Research* (edit. by Bradley, P. B.), 295–332 (Raven, New York, 1975).
- Blaustein, M. P., Johnson, E. M., and Needleman, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2237–2240 (1972).
- Rubin, R. P., *Pharmac. Rev.*, **22**, 389–428 (1970).
- Langer, S. Z., Stefano, F. J. E., and Enero, M. A., *J. Pharmac. exp. Ther.*, **183**, 90–102 (1972).
- Taylor, K. M., and Laverty, R., *J. Neurochem.*, **16**, 1361–1366 (1969).

Foetal thymidine kinase in tumours and colonic flat mucosa of man

We have characterised two forms of deoxythymidine kinase (TK) using antisera prepared against the enzyme partially purified from human colonic mucosa. One of these forms is associated with rapidly proliferating lower colonic crypt cells and the other, with non-dividing surface cells. Antibody to the latter cross reacted with several tumours and placental extracts but not with phytohaemagglutinin (PHA)-stimulated or leukaemic lymphocytes. This cross reactivity of surface cells and colonic tumours supports the concept that surface cells rather than crypt cells are the site of origin of colonic cancer^{1,2}.

Studies in preneoplastic tissues showed a persistent incorporation of labelled deoxythymidine into DNA of the normally quiescent cells of colonic¹⁻³ and gastric⁴ mucosae and cervical epithelium⁵. These observations directed our attention to the enzymes associated with DNA synthesis in these non-dividing cells as well as normal and malignant proliferating tissues. The concomitant increases in DNA synthesis and the pyrimidine salvage enzyme, TK, in proliferating cells and tissues and physiologically stimulated systems^{6,7} focused on this enzyme as a parameter linked to proliferation and malignancy. For example, human colorectal tumours contain more TK than the homologous, normally rapidly proliferating tissue⁸. In the latter, TK activity is found primarily in the lower third of the colonic crypt, the region containing most of the rapidly proliferating cells^{8,9}. Tumour TK differs from the normal enzyme in its response to mercaptans and is more thermolabile in the presence and absence of mercaptans⁸.

These data, as well as the observations that TKs induced by infection with herpesvirus types 1 and 2 are biochemically and antigenically distinct not only from cellular TK but also from each other^{10,11}, suggested that the serological properties of TK could be used to detect and characterise colorectal malignancies. Antisera were prepared in the rabbit, using partially purified TK from total normal colonic mucosa as immunising antigen (Table 1), and screened by Ouchterlony double diffusion technique¹² against cytosol (127,000g supernatant fraction) TKs from normal total colon, colonic surface

and crypt cells, placenta, PHA-stimulated lymphocytes, leukaemic cells and tumours from sites other than the colon.

Both preimmune and immune sera were tested against homologous and heterologous enzymes. Preincubation with preimmune sera did not have any effect on TK activity of any

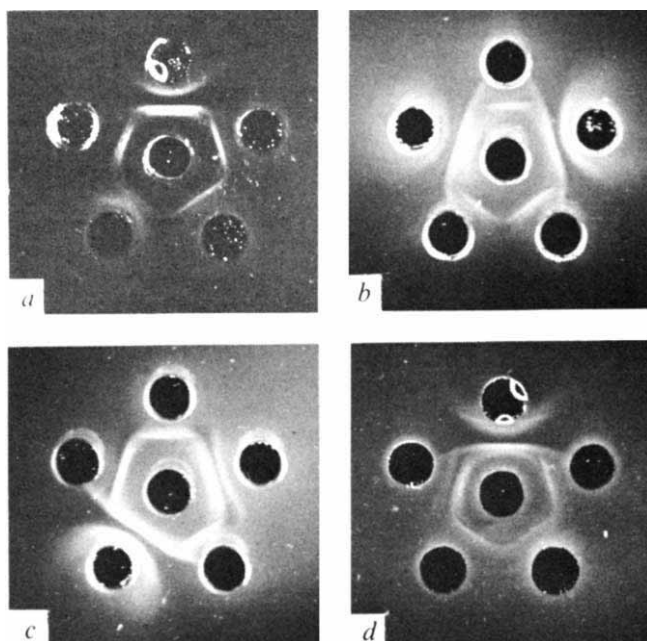


Fig. 1 Double diffusion precipitin reactions in agar gels. Immune serum (0.004 ml) is used in the centre wells and antigen extracts (35–40 µg cytosol protein), in the peripheral wells of micro-Ouchterlony plates (5-mm well spacings, Hyland Laboratories). The peripheral wells are designated as 1, at the top and sequentially numbered in a clockwise direction. In *a* the extracts are 1, total colonic mucosa; 2, adenocarcinoma of the colon (J.G.); 3, colonic surface (tip) cells; 4, colonic lower crypt cells and 5, placenta I. In *b* the extracts are 1, placenta I; 2, total colonic mucosa; 3, osteosarcoma, lung; 4, adenocarcinoma of the rectum (N.I.) and 5, ascites from metastatic adenocarcinoma of the colon (I.R.). In *c* the extracts are 1, adenocarcinoma of the colon (A.S.); 2, ovarian carcinoma; 3, adenocarcinoma of the rectum (C.W.); 4, total colonic mucosa and 5, colonic polyp. In *d* the extracts are 1, total colonic mucosa; 2, adenocarcinoma of the colon (I.R.); 3, colonic polyp; 4, adenocarcinoma of the colon (J.G.) and 5, placenta II.

Table 1 Purification of TK from human colonic mucosa

	Total protein (mg)	Total activity (µU)	Specific activity (µU per mg protein)	Yield (%)
Whole homogenate	9,940	52,682	5.3	
Supernatant (10 ⁵ g)	4,552	45,975	10.1	87.2
(NH ₄) ₂ SO ₄ precipitation (40%)	358	34,117	95.3	64.8
Heat at 50 °C (1 mM TdR)	143	28,629	200.2	54.3
(NH ₄) ₂ SO ₄ precipitation (40%), concentrated on Amicon after dialysis	64	25,446	397.6	48.3
Negative Ca ₃ (PO ₄) ₂ gel	22.7	19,034	838.5	36.1
Sephadex SG 200 (KCl) chromatography	6.9	10,554	1,529.6	20.0
BioGel P-150 chromatography	1.4	6,327	4,519.3	12.0
DEAE-cellulose chromatography	0.3	3,347	11,156.5	6.4

Pooled total mucosal scrapings (140 g frozen wet weight) from autopsy colons of patients with non-involvement of the gastrointestinal tract, obtained through the Tumour Procurement Service of Memorial Hospital, New York, were homogenised in a Waring Blender at low speed with 0.25 M sucrose–0.2 M Tris-HCl (pH 8.2, 25 °C)–0.15 M KCl–0.17 M 2-mercaptoethanol (25:1.5:1:0.5), strained through gauze and centrifuged at 100,000g for 90 min. The pellet was washed and re-extracted twice and the combined supernatant fractions were adjusted to 40% saturation with respect to ammonium sulphate. The precipitate collected by centrifugation at 20,000g for 20 min was redissolved in 0.05 M Tris-HCl–0.25 M sucrose–6 mM 2-mercaptoethanol–1 mM TdR, heated at 50 °C for 5 min, rapidly cooled, centrifuged and reprecipitated with ammonium sulphate. The precipitate collected by centrifugation was dissolved and purified by absorption on calcium phosphate gel, followed by gel filtration in Sephadex SG-200 in the presence of salt and in BioGel 150 in the absence of salt and finally by elution with a linear salt gradient (0.05 M–1.0 M KCl) from DEAE-cellulose. This resulted in a 2,100-fold purification of the enzyme with respect to the activity in the whole homogenate. All steps were carried out at 4–8 °C. TK activity was assayed as before. A unit is defined as the enzyme phosphorylating 1 µmol TdR per min at 37 °C. For immunisation, 1 mg of purified colonic TK in 1.0 ml of 0.05 M Tris–1 mM TdR–6 mM 2-mercaptoethanol was emulsified by mixing with an equal volume of Freund's complete adjuvant and injected subcutaneously into New Zealand white albino male rabbits. This was followed a week later by a similar preparation with incomplete adjuvant. A booster injection with incomplete adjuvant was administered 6 weeks after the initial injection. Blood was collected from the marginal ear vein once a week for about 4 or 5 weeks after the booster.

Table 2 Residual activity of TK in various tissue extracts after preincubation with anti-human colonic TK from rabbits

Source of TK	Control TK activity* (pmol per incubation sample)	Undiluted	% Residual activity†	
			1:10	1:1,000
Partially purified by a single precipitation with ammonium sulphate				
Normal colon	40±2	8	27	37
Adenocarcinoma colonic (J.G.)	66±3	54	67	86
Placenta I‡	68±2	60	77	87
Crude extract				
Ovarian carcinoma	80±4	35	48	65
PHA-stimulated lymphocytes	76±2	95	99	100
Myeloblastic leukaemic leukocytes	85±4	95	98	100

Cytosol TKs were prepared from surgical samples after homogenisation in the buffer described in Table 1, with a Potter–Elvehjem homogeniser and centrifugation at 127,000g for 60 min; the supernatant could be stored at –20 °C for more than 2 months with little loss in activity and at –80 °C for more than a year. Lymphocytes and leukaemic cells were washed with phosphate-buffered saline, pelleted, resuspended in a minimal volume of distilled water and lysed by repeated freezing and thawing. The lysed cell suspension was adjusted to the same concentration of homogenisation medium and gently homogenised as were the surgical samples. TK activity was assayed as before⁸.

*Figures are pmol per incubation sample. Control activity is the average of the enzyme activity in preparations preincubated with preimmune serum. The latter had no effect on the TK activity of any of the preparations tested. TK activity was assayed as before⁸.

†Values signify percentage residual activity obtained with antisera relative to activity with preimmune serum. Various dilutions of both pre-immune serum and serum (No. 317) of rabbit immunised with human colonic TK were preincubated overnight at 4 °C with equal volumes (0.20 ml containing 250–300 µg protein) of either dialysed preparations of TK partially purified by ammonium sulphate precipitation or crude 127,000g supernatant fractions. Three samples of the supernatant containing 50–60 µg antigen protein were removed and assayed for TK activity.

‡Placentae used in all these studies were flushed for at least 15 min with physiological saline through the umbilical artery. A portion of the foetal placenta free of maternal elements was removed and homogenised as described for surgical samples.

of the preparations examined. Antisera, which markedly inhibited homologous colonic TK were much less effective in neutralising the activity of enzymes from colonic adenocarcinoma, placenta (two samples, I and II) and ovarian carcinoma and did not inhibit the enzyme from either PHA-stimulated lymphocytes or myeloblastic leukaemic leukocytes (Table 2). The latter two preparations did not react with antisera to produce double diffusion precipitin lines in contrast to the extracts of total colonic mucosae, colonic crypt cells, colonicsurface (tip) cells, placenta and tumours of diverse origin (Fig. 1). The precipitin lines were absent when supernatant from total colonic mucosa preincubated with undiluted antiserum was used as antigen, suggesting that these lines are essentially attributable to TK–antibody reactions. There were cross reactions between colonic TK and heterologous enzymes from placenta and colonic tumours (Fig. 1a and d), osteogenic sarcoma (lung) and placenta (Fig. 1b), rectal carcinoma and colonic polyp (Fig. 1c) and total mucosa from small intestines, suggesting partial antigenic identity of the enzymes in these preparations. There seemed to be cross reactions between TK from ascitic cells (metastatic colonic adenocarcinoma) and the enzymes from placenta and rectal adenocarcinoma (Fig. 1b). Precipitin patterns similar to those presented here for tumours were also observed in a breast carcinoma, a myxoliposarcoma, a gastric carcinoma and a cervical carcinoma.

In mammalian cells, there are multiple forms of TK^{13–15}, the relative proportions of which vary with the origin and the metabolic state of the tissue. The different precipitin patterns in the rapidly proliferating tissues examined could be a reflection of quantitative and/or qualitative differences in the isozymes present. The biphasic nature of the thermal inactivation observed in crude cytosol fractions⁸ as well as in the partially purified colonic TK preparations suggests the presence of more than one form of the enzyme. Preliminary studies also indicate that there are at least three electrophoretic forms of TK in some of these tissues, and in the 2,100-fold purified TK used as antigen. In the latter, TK activity was associated with the three protein-staining bands which constituted about 90% of the total protein. There seems to be at least one form of TK in colonic tumours, polyps and placenta which is antigenically similar, but not identical, to that of normal colonic TK, most likely the form found in the surface cells.

Although TK activities from placenta and a colonic adenocarcinoma (J.G.) were inhibited to about the same extent (Table 2), the enzymes do not seem to be serologically identical (Fig. 1d). There is a similarity between these placental and

tumour antigens but not the complete identity which has been reported for isozymes of human alkaline phosphatase¹⁶. TK with the electrophoretic migratory pattern characteristic of the enzyme found in the early stages of development of human foetal liver has been observed in malignant tumours from various sites^{15,17,18}. Enzymes with similar migration, however, are not necessarily antigenically related, for example, from cervical carcinoma and the virus-specific enzyme induced by the epidemiologically associated herpesvirus type 2 (ref. 19). The similarity between TKs from placenta and tumours from various sources is reminiscent of the appearance of embryonal antigens in murine²⁰ and human²¹ cancer cells.

The data presented here suggest that TK of colonic tumour is serologically more similar to the enzyme from surface cells than that from lower crypt cells. This would be consistent with the hypothesis that colonic tumours arise from surface epithelial cells rather than from the depths of the crypts^{1,2}.

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JOSEPHINE S. SALSER
M. EARL BALIS

Laboratory of Cell Metabolism,
Memorial Sloan–Kettering Cancer Center,
New York, New York 10021

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- 1 Cole, J. W., *Hospital Practice*, **8**, 123–130 (1973).
- 2 Lipkin, M., *Cancer*, **34**, 878–888 (1974).
- 3 Deschner, E., and Lipkin, M., *J. natn. Cancer Inst.*, **44**, 175–185 (1970); *Cancer*, **35**, 413–418 (1975).
- 4 Winawer, S. J., and Lipkin, M., *J. natn. Cancer Inst.*, **42**, 9–17 (1969).
- 5 Wilbanks, G. D., Richart, R. M., and Terner, J. Y., *Am. J. Obstet. Gynec.*, **98**, 792–799 (1967).
- 6 Roth, J. S., in *Protein Metabolism and Biological Function* (edit. by Bianchi, C. P., and Hilf, R.), 141–219 (Rutgers University Press, New Brunswick, 1970).
- 7 Kit, S., Leung, W.-C., and Trkula, D., in *Control Processes in Neoplasia* (edit. by Mehlman, M. A., and Hanson, R. W.), 103–145 (Academic, New York, 1974).
- 8 Salsar, J. S., and Balis, M. E., *Cancer Res.*, **33**, 1889–1897 (1973); *Cancer*, **34**, 889–895 (1974).
- 9 Troncale, F., Hertz, R., and Lipkin, M., *Cancer Res.*, **31**, 463–467 (1971).
- 10 Buchan, A., and Watson, D. H., *J. gen. Virol.*, **4**, 461–463 (1969).
- 11 Thousless, M. E., *J. gen. Virol.*, **17**, 307–315 (1972).
- 12 Ouchterlony, O., *Progr. Allerg.*, **5**, 1–78 (1958).
- 13 Kit, S., Leung, W.-C., Jorgenson, G. N., Trkula, D., and Dubbs, D. R., *Cold Spring Harb. Symp. quant. Biol.*, **34**, 703–715 (1974).
- 14 Adler, R., and McAusland, B. R., *Cell*, **2**, 113–117 (1974).
- 15 Taylor, A. T., Stafford, M. A., and Jones, O. W., *J. biol. Chem.*, **247**, 1930–1935 (1972).

- ¹⁶ Fishman, W. H., *Adv. Enzyme Regul.*, **11**, 293–321 (1973).
¹⁷ Stafford, M. A., and Jones, O. W., *Biochim. biophys. Acta*, **277**, 439–442 (1972).
¹⁸ Bull, D. L., Taylor, A. T., Austin, D. M., and Jones, O. W., *Virology*, **57**, 279–284 (1974).
¹⁹ Rawls, W. E., *et al.*, *Cancer Res.*, **34**, 362–366 (1974).
²⁰ Stonehill, E. H., and Bendich, A., *Nature*, **228**, 370–372 (1970).
²¹ Klavins, J. V., Mesa-Tejada, R., and Weiss, M., *Nature new Biol.*, **234**, 153–154 (1971).

Immunofluorescent localisation of human pregnancy-specific β_1 -globulin in placenta and chorioepithelioma

VARIOUS immunological methods have been used to define the existence on plasma of several pregnancy-associated macromolecules^{1–3}, of which between three and five are now well recognised in humans^{4,5}. The cells and tissues responsible for their synthesis, however, remain to be clearly defined, although, of the two pregnancy-associated β_1 -globulins, one was recently detected in the sera of patients bearing trophoblastic tumours⁶. This particular β_1 -globulin does not resemble any known hormone, including chorionic gonadotropin⁷, but seems to be identical with the pregnancy-specific β_1 -glycoprotein of Bohn⁸ and the PAPP-C described by Lin *et al.*⁹. The placenta has been suggested as the site of its synthesis^{8,9}. Using indirect immunofluorescence, we have now located this β_1 -globulin in human placenta and chorioepithelioma of the uterus, where we believe it is synthesised.

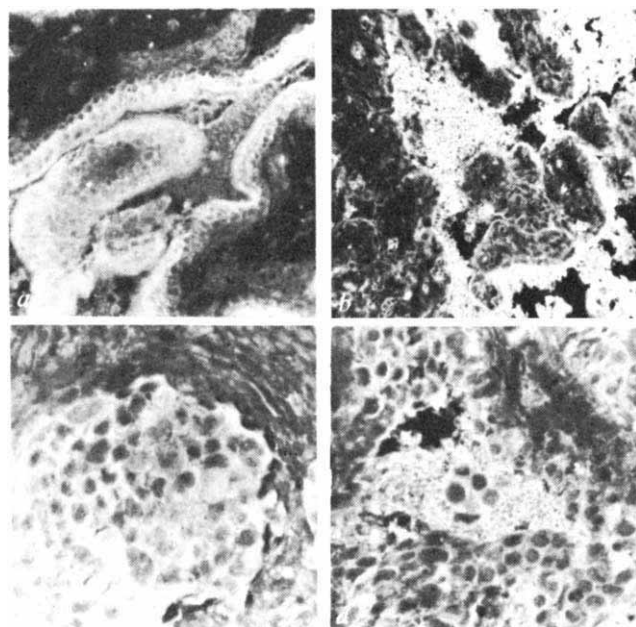


Fig. 1 *a*, Section of placental chorion (8-week gestation) treated with anti- β_1 -globulin and anti-RGGFI, showing specific fluorescence in cytoplasm of cytotrophoblastic (Langhans) and syncytiotrophoblastic cells ($\times 31$). *b*, Section of the placenta (40-week gestation) treated with anti- β_1 -globulin and anti-RGGFI, showing specific fluorescence in residual trophoblastic cells and in fibrin islands ($\times 31$). *c* and *d*, Sections of different chorioepitheliomas, showing specific fluorescence in cytoplasm and membranes of Langhans cells as well as in fibrin islands (also treated, $\times 52$).

Samples of placenta were obtained from abortions, carried out at 6–12 weeks of gestation, or after normal birth. Chorioepitheliomas were obtained from affected patients at the time of hysterectomy. For controls, specimens of other organs were obtained at autopsy, 3–6 or 8–16 h after death, from patients with either chorioepitheliomas (without metastases) or non-malignant disease.

Tissue specimens (diameter 3–5 mm) were fixed in cold absolute ethanol with glacial acetic acid (99:1 v/v)¹⁰ and subsequently embedded in paraffin and sectioned¹¹. Anti- β_1 -globulin was prepared as before⁸. Antibodies to anti- β_1 -globulin and anti-albumin of human serum (anti-HSA) were raised in rabbits and then purified by the glutaraldehyde-immunosorbent technique¹². Human albumin was chosen as the serum protein reference of nonspecific absorption because it is not produced by trophoblastic cells. The indirect immunofluorescence technique was used with donkey monospecific antibodies for rabbit γ -globulin labelled with fluorescent isothiocyanate (anti-RGGFI). Selected fields were photographed (ML-2B microscope) and subsequently stained with haematoxylin and eosin and mounted in glycerol buffer to facilitate comparison with definitively identifiable structures.

To ensure that the fluorescence exhibited by the anti- β_1 -globulin sera was specific for the storage sites in the placenta and chorioepitheliomas, and to distinguish between specific and nonspecific uptake of the antibodies, the following controls were effected: (1) incubation of some sections only with anti-RGGFI; (2) use of anti- β_1 -globulin neutralised with serum from pregnant women; (3) use of anti-HSA and anti-RGGFI on some sections, excluding the liver.

Specific immunofluorescence was found in placenta and chorioepitheliomas but not in lung, muscle, spleen, brain, pancreas, gastrointestinal tract or liver (when there was no metastasis or tumour). It was associated with the cytotrophoblast and syncytiotrophoblast of the chorion in immature placenta (Fig. 1*a*) and with residual trophoblast and fibrin islands, perhaps containing necrotic trophoblastic cells, in the term placenta (Fig. 1*b*). In chorioepithelioma, specific fluorescence was also associated with giant trophoblastic cells (Langhans cells) or fibrin islands (Fig. 1*c* and *d*).

The control experiments with 8-week gestation placental tissue and sections of liver after treatment with anti-HSA and anti-RGGFI showed little or no fluorescence.

We suggest that production of this particular β_1 -globulin may start in the Langhans cells and continue in the syncytiotrophoblast of the normal chorion and in the trophoblast cells of chorioepitheliomas, where there may be less biosynthetic activity and secretory capacity.

Y. S. TATARINOV
D. M. FALALEEVA
V. V. KALASHNIKOV
B. O. TOLOKNOV

Department of Biochemistry,
Immunochemical Laboratory for Research on
Malignant and Embryonal Tissues,
Second Moscow Medical Institute,
Moscow G-435, USSR

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- ¹ Olivelli, P., and Ruggier, P., *Minerva. ginec.*, **10**, 458–460 (1958).
² MacLaren, J. A., Thorne, R. D., Poby, C. C., and Reid, D. E., *Am. J. Obstet. Gynec.*, **78**, 939–946 (1959).
³ Hirschfeld, J., and Sodenberg, U., *Nature*, **187**, 332–333 (1960).
⁴ Hofmann, R., Friemal, H., Behm, E., and Brock, J., *Arch. Gynäk.*, **208**, 255–265 (1970).
⁵ Lin, T.-M., Halbert, S. P., Kiefer, D., Spellasy, W. N., and Gall, S., *Am. J. Obstet. Gynec.*, **118**, 223–236 (1974).
⁶ Tatarinov, Yu. S., *et al.*, *Int. J. Cancer*, **14**, 548–554 (1974).
⁷ Tatarinov, Yu. S., Nikoulina, D. M., and Mesnyankina, N. V., *Labor. Delo*, **9**, 547–549 (1974).
⁸ Tatarinov, Yu. S., and Masyukevich, V. N., *Bull. exp. Biol. Med. U.S.S.R.*, **69**(6), 66–69 (1970).
⁹ Bohn, H., *Arch. Gynäk.*, **210**, 440–457 (1971).
¹⁰ Hamashima, J., Harter, J. G., and Coons, A. H., *J. Cell Biol.*, **20**, 271–279 (1964).
¹¹ Sainte-Marie, G., *J. Histochem. Cytochem.*, **10**, 250–256 (1962).
¹² Avrameas, S., and Ternynck, T., *Immunochimistry*, **6**, 53–67 (1969).

Immunological and biochemical characterisation of type C viruses isolated from cultured human AML cells

TYPE C viruses are associated in several species with naturally occurring malignancy and have been isolated from a variety of species including several non-human primates. The ability to specify inter-viral relationships is crucial for evaluating the degree of relatedness of new isolates to known viruses, especially those which are potentially associated with human malignancy. Recent studies of Gallagher and Gallo¹ indicated the presence of a type C virus in cultured cells from a patient with acute myelogenous leukaemia (AML) (patient A.S.). Viruses from this culture have been propagated in homologous and heterologous fibroblastic cells² in sufficient quantity to be characterised in detail.

Recently, immunological and molecular hybridisation methodology has been applied to the type-specific differentiation of known primate viruses³⁻⁸. The viruses that can be distinguished include three members of the related woolly monkey (SSV-1)-gibbon ape group and three members of the endogenous baboon virus-RD114 group (Table 1). In investigating the relationship of the AML virus isolate to known type C viruses using the above methodology, certain of our initial observations were previously recorded as "personal communication"². It is our purpose here to amplify these findings. The data reported here were derived from virus produced by secondary fibroblastic cells² after infection with virus from primary leukocyte cultures of the first peripheral blood sample¹.

The AML virus (HL23V), propagated in either canine thymus or human cells was initially found to contain proteins of both primate virus groups based on complement-fixation tests and radioimmunoassays which are specific for woolly monkey-gibbon virus p30 and RD114-baboon virus p30, respectively. Growth of the HL23 virus in normal rat kidney (NRK) cells, which are relatively non-permissive

for viruses of the RD114-baboon group, yielded a virus which only showed immunological reactivity characteristic of the woolly monkey-gibbon virus group.

The suggestion that two viruses were present in infected human and dog cultures was confirmed by detection of viral RNA using complementary ³H-DNA (cDNA) prepared in the endogenous reverse transcriptase reaction. Uninfected cultures were negative in these assays. On the basis of the extent of its reaction using S1 nuclease, one of the viruses was indistinguishable from the woolly monkey virus (Fig. 1a). The woolly cDNA preparation hybridised to a lesser extent with the two gibbon (GALV) viruses and gave no hybridisation with cultures infected with the baboon or RD114 viruses or a culture replicating endogenous rat virus. The gibbon (SFMC) cDNA used gave the expected reciprocal reaction, namely a partial reaction with woolly monkey and AS virus-infected cell RNA (Fig. 1b). In view of the >95% reaction it was not surprising to find that thermal dissociation profiles of woolly cDNA and woolly or HL23V RNA had midpoints (*T_m*) within 1 °C, whereas in the same conditions (hydroxyapatite chromatography) woolly monkey and gibbon heterologous DNA-RNA hybrid molecules showed a ΔT_m of 6.5 °C compared with homologous reactants (data not shown).

The second virus present has sequences indistinguishable from the endogenous baboon virus isolated from *Papio cynocephalus* (Fig. 1c). The cDNA preparation used distinguished this virus from a highly related endogenous virus isolated from the baboon species *Papio hamadryas*, which reached saturation at 80% of the homologous reaction and RD114: maximal reaction ~20%. The cDNA did not hybridise with human cell RNA including that of the cell line (uninfected) used to propagate the virus. No cross hybridisation was obtained with RNA from cells producing the woolly monkey virus. RD114 cDNA (Fig. 1d) gave the expected reciprocal reaction with baboon and AS viruses, namely ~20% at saturation. Note also that AS virus grown in rat cells did not hybridise with baboon or RD114 cDNAs, consistent with the immunological data indicating the presence of only the woolly monkey viral p30 in this culture. The relationship between the two baboon viruses seems closer than that of the woolly monkey-gibbon group based

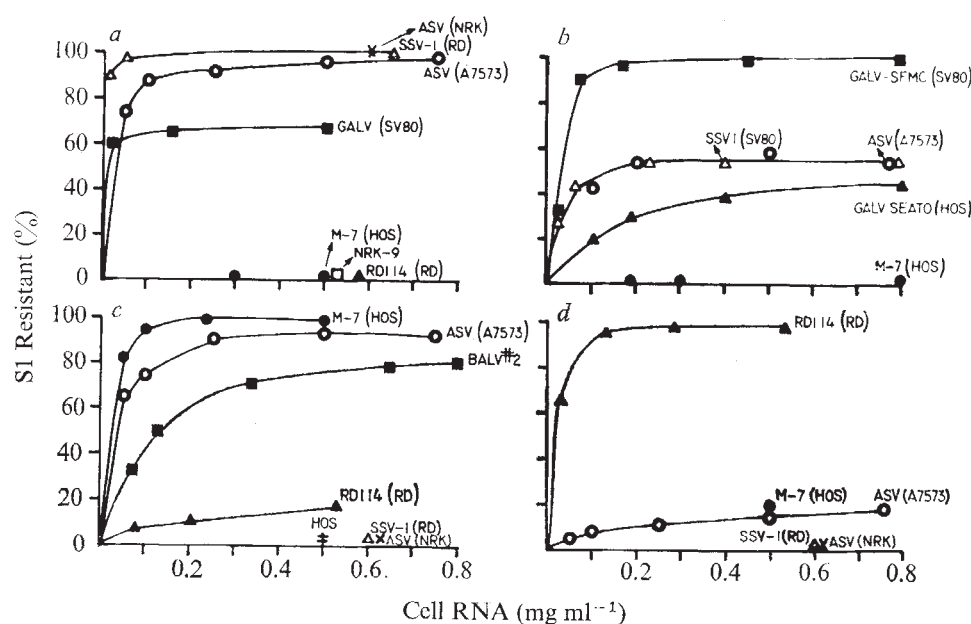


Fig. 1 ³H-DNA transcripts (about 10³ c.p.m. per assay) of woolly monkey virus (SSV-1) which represents the helper virus (SSAV) present in vast excess, the SFMC isolate of GALV, the baboon isolate of *Papio cynocephalus* (M-7), and the endogenous cat virus (RD114) were annealed with increasing amounts of cellular RNA. Incubation was at 67 °C for 18 h and mixtures were analysed by S1 nuclease digestion according to previously described methods⁹. The cDNAs used represent 60–70% of the various viral genomes based on resistance of labelled RNA to RNase digestion after annealing with excess cDNA. Values are normalised to 100% for the homologous reactants, actual values were 90% or greater for each of the four homologous sets of reactants. The cells in which the various viruses were grown are given in parentheses. NRK, Normal rat kidney; RD human rhabdomyosarcoma cell line; A7573,

canine thymus cell line; SV80, human cell transformed by SV40; HOS, human osteogenic sarcoma cell line; NRK-9, rat cell producing endogenous rat virus; BALV No. 2, baboon virus from *P. hamadryas* grown in canine thymus cells. The data shown in a are for GALV-SFMC, similar results were obtained with GALV-SEATO. HL23V virus grown in human cells gave a similar hybridisation pattern to that shown for the virus grown in canine cells. a, ³H-DNA SSV-1 (RD); b, ³H-DNA GALV-SFMC (SV80); c, ³H-DNA M7 (HOS); d, ³H-DNA RD114 (RD).

Table 1 Primate and related type C viruses distinguished by type-specific molecular hybridisation and immunoassays

	Virus	Origin
A (1)	Woolly monkey (SSAV-1)	Fibrosarcoma ¹⁰
	Gibbon ape (GALV-SFMC)	Lymphosarcoma ¹¹
	Gibbon (GALV-SEATO)	Granulocytic leukaemia ¹²
B (1)	RD114	Endogenous cat virus ¹³
	Baboon (BALV-M7-Papio cynocephalus)	Placenta ¹⁴
	Baboon (BALV No. 2-Papio hamadryas)	Lymph node of lymphomatous baboon ¹⁵

Viruses of the A group are highly related as are those of the B group. The assays described in this report show no cross reactivity between groups. B-group viruses are endogenous in their species of isolation¹⁶, while A-group viruses are not represented in cellular DNA of their species of isolation¹⁷. A-group viruses are believed to have originated in the remote past from ancestors of the genus *Mus*^{18,19}.

SFMC: San Francisco Medical Centre; SEATO: laboratory in Bangkok, Thailand.

on thermal dissociation data. Homologous heteroduplexes of the baboon viruses gave T_m s 2 °C higher than heterologous reactants. The HL23 virus, as expected from reaction extent, was indistinguishable by thermal stability criteria from the *Papio cynocephalus* virus (within 1 °C).

The distinct components of the HL23 virus preparation were further analysed in type-specific radioimmunoassays for low molecular weight viral structural polypeptides³. The HL23 virus and each of the woolly monkey-gibbon viruses showed complete cross reactivity in the woolly monkey virus p30 assay. In the woolly monkey p12 assay (Fig. 2a), HL23 virus and woolly monkey virus showed identical slopes and

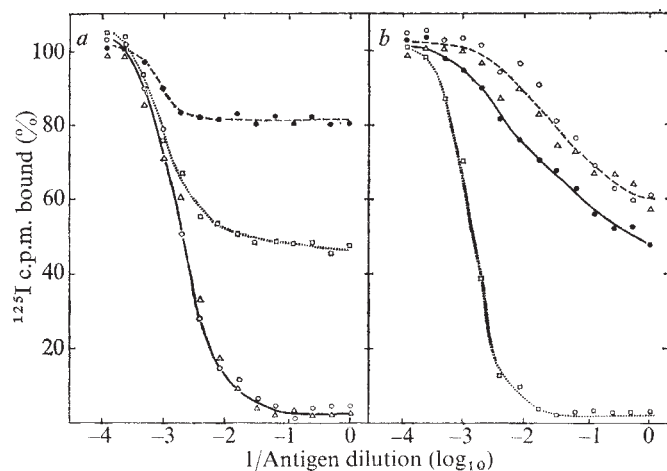


Fig. 2 Analysis of the HL23V virus in competition immunoassays for woolly monkey and gibbon ape virus p12 polypeptides³. Viruses were disrupted by incubation at 37 °C for 30 min in 1% Triton and were tested at serial twofold dilutions for ability to compete with ¹²⁵I-labelled p12 for binding limiting amounts of antiserum. Reaction mixtures contained 0.01 M Tris, 0.1 M NaCl, 0.15 M EDTA, 0.1% Triton X-100, and 1% bovine serum albumin (pH 7.8) in a volume of 0.7 ml. Antiserum and unlabelled competing antigen were incubated at 37 °C for 1 h; ¹²⁵I-labelled antigen was added, and the incubation continued for 3 h at 37 °C and a further 18 h at 4 °C. After addition of 0.025 ml undiluted swine anti-goat IgG to each tube, the mixture was incubated for 3 h at 4 °C, centrifuged at 2,500 r.p.m. for 15 min and the resulting precipitate measured for radioactivity. Viruses tested included the woolly monkey virus¹⁰ (○), SFMC gibbon ape virus¹¹ (□), SEATO gibbon ape virus¹² (●), and the HL23V virus^{1,2} (△). a, Competition immunoassay using antiserum against woolly monkey virus to precipitate ¹²⁵I-labelled woolly monkey virus p12; b, competition immunoassay using antiserum against SFMC gibbon ape virus to precipitate gibbon ape virus p12.

final reaction extents (complete displacement of the iodinated woolly monkey p12), while the two gibbon viruses were easily distinguished from the above viruses by these criteria. In the SFMC gibbon virus p12 assay, only the SFMC virus competed efficiently (Fig. 2b).

In analogous immunoassays for baboon-RD114 structural polypeptides (Fig. 3) both the AS virus and the endogenous virus of *P. cynocephalus* reacted efficiently in the *P. cynocephalus* virus p15 assay, but competed poorly in the RD114 p15 assay. In contrast, the *P. hamadryas* virus was poorly reactive in both p15 assays. RD114 competed completely in the homologous RD114 p15 assay but not in the *P. cynocephalus* p15 assay. In other studies the gp70s of the woolly monkey⁴ and baboon components of the HL23 virus preparation were indistinguishable from the gp70s of the prototype woolly monkey virus and *P. cynocephalus* virus in competition assays for these polypeptides (data not shown).

To substantiate further the presence of two distinct viruses, HL23 virus preparations from canine thymus cells were mixed in separate tubes with neutralising antibody specific for baboon and woolly monkey virus and the mixtures added to human (RD) cells. At 3 weeks, cultures infected with HL23 virus plus anti-baboon serum contained

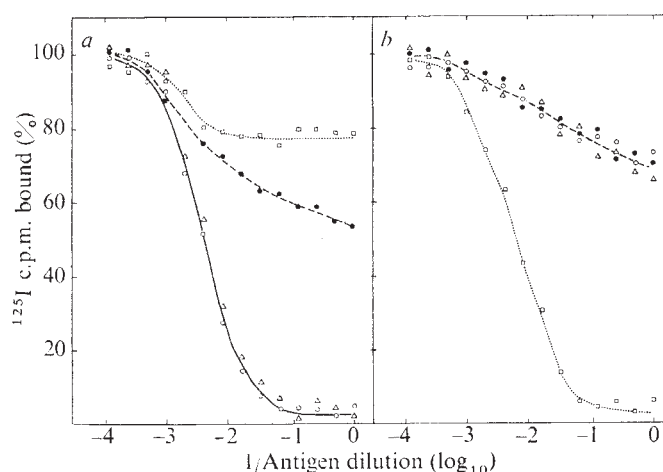


Fig. 3 Analysis of HL23V virus in competition immunoassays for the baboon virus and RD114 p15 (ref. 5). Viruses were assayed as described in the legend to Fig. 2 and included the *P. cynocephalus*¹⁴ (○) and *P. hamadryas*¹⁵ (●) baboon viruses; RD114¹³ (□); and the AS virus^{1,2} (△). a, Competition immunoassay using an antiserum against *P. cynocephalus* baboon virus to precipitate ¹²⁵I-labelled *P. cynocephalus* baboon virus p15; b, competition immunoassay using an antiserum against RD114 to precipitate ¹²⁵I-labelled RD114 p15.

only woolly monkey virus p30 and those infected with HL23 virus plus anti-woolly serum contained only baboon virus p30. It was also observed that passage of virus from diluted supernatant fluids readily selected for the woolly monkey virus component as might be expected from the severalfold excess of woolly virus proteins in the mixture.

The present studies thus show that HL23 virus preparations contain two distinct viruses; one indistinguishable from the woolly monkey virus, the second indistinguishable from an endogenous virus of the baboon species, *Papio cynocephalus*.

These data pose critical questions concerning the origin of the HL23 viruses. The high degree of relatedness to two known viruses constitutes argument for accidental contamination, yet other data (refs 1, 2 and refs quoted therein) have suggested the possibility of direct origin of the viruses from fresh cells of the patient. The important question is that of the relationship of these viruses to human disease,

an issue which is under active investigation in several laboratories.

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HIROMI OKABE
RAYMOND V. GILDEN*
MASAKAZU HATANAKA

Flow Laboratories, Inc.,
Rockville, Maryland 20852

JOHN R. STEPHENSON
ROBERT E. GALLAGHER
ROBERT C. GALLO
STEVEN R. TRONICK
STUART A. AARONSON

National Cancer Institute,
National Institutes of Health
Bethesda, Maryland 20014

Received October 31, 1975; accepted February 3, 1976.

* Present address: Frederick Cancer Research Center, Frederick, Maryland 21701.

- ¹ Gallagher, R. E., and Gallo, R. C., *Science*, **187**, 350–353 (1975).
- ² Teich, N. M., *et al.*, *Nature*, **256**, 551–555 (1975).
- ³ Tronick, S. R., Stephenson, J. R., Aaronson, S. A., and Kawakami, T. G., *J. Virol.*, **15**, 115–120 (1975).
- ⁴ Hino, S., Stephenson, J. R., and Aaronson, S. A., *J. Immunol.*, **115**, 922–927 (1975).
- ⁵ Stephenson, J. R., Reynolds, R. K., and Aaronson, S. A., *J. Virol.*, **17**, 374–384 (1976).
- ⁶ Gilden, R. V., *et al.*, *Intervirology*, **2**, 360–365 (1973/74).
- ⁷ Benveniste, R. E., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3316–3320 (1973).
- ⁸ Todaro, G. J., *et al.*, *Virology*, **67**, 335–343 (1975).
- ⁹ Okabe, H., Gilden, R. V., and Hatanaka, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3923–3927 (1973).
- ¹⁰ Theillen, G. H., Gould, D., Fowler, M., and Dungworth, D., *J. natn. Cancer Inst.*, **47**, 881–889 (1971).
- ¹¹ Kawakami, T. G., *et al.*, *Nature new biol.*, **235**, 170–171 (1972).
- ¹² Kawakami, T. G., Buckley, P. M., *Transplant. Proc.*, **6**, 193–196 (1974).
- ¹³ McAllister, R. M., *et al.*, *Nature new biol.*, **235**, 3–6 (1972).
- ¹⁴ Benveniste, R. E., *et al.*, *Nature*, **248**, 17–20 (1974).
- ¹⁵ Goldberg, R. J., Scolnick, E. M., Parks, W. P., Yakovleva, L. A., and Lapin, B. A., *Int. J. Cancer*, **14**, 722–730 (1974).
- ¹⁶ Benveniste, R. E., Heinemann, R., Wilson, G. L., Callahan, R., and Todaro, G. J., *J. Virol.*, **14**, 56–57 (1974).
- ¹⁷ Scolnick, E. M., *et al.*, *J. Virol.*, **13**, 363–369 (1974).
- ¹⁸ Lieber, M. M., *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2315–2319 (1975).
- ¹⁹ Wong-Staal, F., Gallo, R. C., and Gillespie, D., *Nature*, **256**, 670–672 (1975).

Characterisation of a virus (HL23V) isolated from cultured acute myelogenous leukaemic cells

THE isolation of a type C RNA tumour virus (HL23V) from cultured human acute myelogenous leukaemia peripheral blood leukocytes has recently been reported^{1,2}. The reverse transcriptase of this putative human oncornavirus was found¹ to be immunologically related to the reverse transcriptase of the simian sarcoma virus, type 1 (SSV-1), the gibbon ape lymphoma virus (GALV), and to a small extent in secondary culture, RD114 (ref. 2). The spontaneously released virus was shown to be infectious for several cell cultures, including a human rhabdomyosarcoma cell line, A204 (ref. 2). This culture produced virus in sufficient quantities to permit biochemical studies. Here we describe the characterisation of the virus(es) grown in this culture. We find that the particles produced by the A204 culture infected with HL23V are a mixture of two viruses that are indistinguishable immunologically and in nucleotide sequence from two known non-human primate viruses, the baboon endogenous virus (BV-M7)^{3,4} and the woolly monkey virus (SSV-1)⁵.

Our findings complement those reported by Okabe *et al.*⁶ elsewhere in this issue. These investigators looked for similarities with known oncornaviruses by molecular hybridisation with the cytoplasmic RNA from various cell lines infected with HL23V and by antigenic analysis using the type-specific p12 and p15 antigens. Our investigation emphasised molecular hybridisations with the RNA found in the particles released into the supernatant by A204(HL23V)

and our antigenic analysis focused on the major structural antigens (p30 and gp45 of SSV and the p28 of BV-M7).

The A204(HL23V) culture produced high titres of particles that were found by ³H-uridine labelling to possess a characteristic oncornavirus buoyant density of 1.16 g ml⁻¹. Simultaneous detection⁷ assay of the culture supernatants demonstrated that the particles encapsulated 70S RNA and reverse transcriptase. The ³H-DNA synthesised in simultaneous detection reactions and sedimenting as 70S complementary DNA (cDNA)-RNA complexes served as the source of the cDNA probe used in all the hybridisation experiments described here.

The reverse transcriptase from A204(HL23V) culture supernatants was examined for relatedness to the SSV enzyme. In contrast to the report of Gallagher and Gallo¹ (which indicated greater relatedness) but in agreement with Teich *et al.*², the results demonstrated (data not shown) that antisera prepared against the SSV reverse transcriptase was capable of inactivating the reverse transcriptase activity of HL23V particles only to about 60%. This partial inhibition of enzyme activity suggested the presence of a second virus containing an antigenically unrelated enzyme. To identify the unknown component in the HL23V particles, a search was instituted among known animal oncornaviruses using both immunological and molecular hybridisation techniques. The possibilities were quickly narrowed to the RD114/CCC baboon endogenous virus group.

Figure 1a and b shows hydroxyapatite temperature gradient elution profiles of viral cDNAs prepared from SSV and BV-M7 annealed to total RNA from HL23V particles. The extent of hybridisation and the thermal stability indicate that the HL23V particles contain the complete information of both the simian sarcoma virus (SSV) and the baboon endogenous virus isolated from *Papio cynocephalus* (BV-M7).

To determine whether all of the genetic information of HL23V can be accounted for by these two viruses, the reciprocal hybridisations were performed. In these experi-

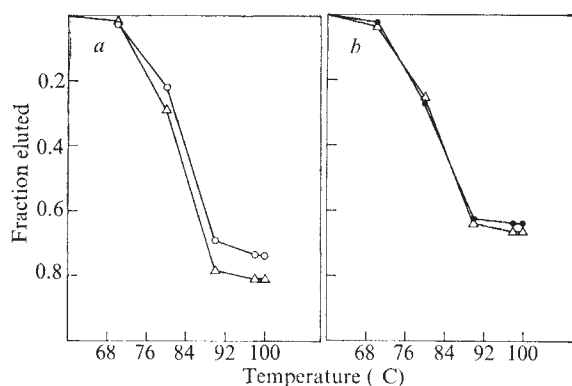


Fig 1 Hybridisation of ³H-DNA transcripts of SSV-1 (a) and BV (M7 isolate) (b) to HL23V viral RNA. SSV-1 and BV were obtained from Pfizer, Inc. and were derived from sucrose density gradient-banded tissue-culture supernatants of a chronically infected human lymphoblastoid cell line (NC-37) and baboon kidney-canine thymus coculture (BKCT), respectively. HL23V virus was prepared from high speed pellets of 2-d-old media from HL23V-infected human rhabdomyosarcoma cell cultures. ³H-DNA probes for each of the viruses were isolated from the 60–70S RNA-DNA hybrids of standard large scale simultaneous detection assays. Hybridisation reactions were set up between the various probes (500–1,000 c.p.m. per assay) and viral RNAs (0.2–1.0 μg) in 20-μl volumes in sealed siliconised glass tubes in 0.8 M phosphate buffer (pH 6.8) 0.1% SDS and 10 mM EDTA. After heating at 100 °C for 1 min, the reactions were incubated at 68 °C for 20 h (*C₀t* > 2). The reactions were analysed by thermal elution hydroxyapatite chromatography. Fractions of the total radioactivity eluted above 60 °C were plotted as a function of temperature. ○, SSV-1 RNA; ●, BV RNA; △, HL23V RNA.

Table 1 Analysis of HL-23 for SSV genomic content by competition hybridisation

Competing RNA	Competing RNA ^{125}I -SSV RNA	Resistance of ^{125}I -SSV RNA %
None	—	72
SSV-1	1,500	3
GALV	1,200	15
MuLV-R	1,800	75
Mouse 18S+20S	450	74
HL-23	2,300	6

Hybridisation reactions (5.5 μl) were performed as described in Fig. 2 and contained 0.11 ng ^{125}I -SSV RNA (1.2×10^8 c.p.m. μg^{-1}), 0.68 ng ^3H -SSV cDNA (2×10^3 c.p.m. μg^{-1}) and 0.5–0.25 μg of the indicated competing RNA. Following incubation at 68 °C for 48 h, the reactions were diluted with 0.01 M Tris-HCl (pH 8.0) 0.4 M NaCl, 0.01 M EDTA and divided into four equal aliquots. RNase A (25 U ml^{-1}) and RNase T₁ (5 U ml^{-1}) were added to two aliquots and the samples incubated at 37 °C for 1 h. Nuclease resistance was the ratio of acid-precipitable ^{125}I in the samples with and without ribonuclease. Recovery of input acid-precipitable ^{125}I was greater than 90%. SSV RNA was iodinated by the procedure of Commerford⁸ as modified by Tereba and McCarthy⁹. SSV RNA was isolated from virions by disruption with SDS, Pronase treatment, rate sedimentation in a sucrose-SDS gradient and equilibrium density gradient centrifugation in potassium iodide. Viral RNAs from GaLV and MuLV-R were isolated by similar procedures excepting the KI gradient. HL23 VRNA was total RNA from purified virus and the mouse 18S and 20S RNAs were extracted from purified ribosomal subunits from NIH/3T3 tissue culture cells. ^3H -SSV cDNA was synthesised from SSV RNA and oligo(dT) with AMV DNA polymerase in the presence of 0.1 mg μl^{-1} actinomycin D and 0.5 mg ml^{-1} distamycin A. The reaction contained TTP, dATP and dGTP at 1 mM each and ^3H -dCTP (25 Ci mmol^{-1}) at 0.05 mM. This SSV cDNA protected ^{125}I -SSV RNA 33%, 75% and 87% from ribonuclease digestion at molar input ratios of 0.4, 3 and 15, respectively (cDNA-RNA).

ments, cDNA probe was synthesised with HL23V particles and was annealed to RNAs from SSV-1 or BV-M7 or both. Figure 2 shows that the HL23V cDNA hybridised 37% and 44% to the BV-M7 and SSV-1, respectively. These individual hybridisations were additive as demonstrated by the complete complexing of HL23V cDNA to a mixture of

Fig. 2 Reconstruction hybridisations. HL-23 ^3H -DNA probe was hybridised to SSV and BV RNAs individually and in combination. ○, SSV RNA; △, BV RNA; ●, SSV and BV RNAs.

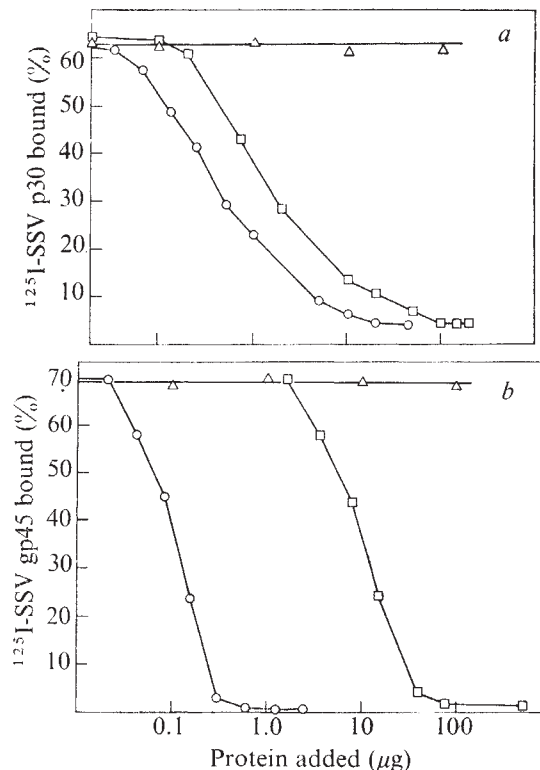
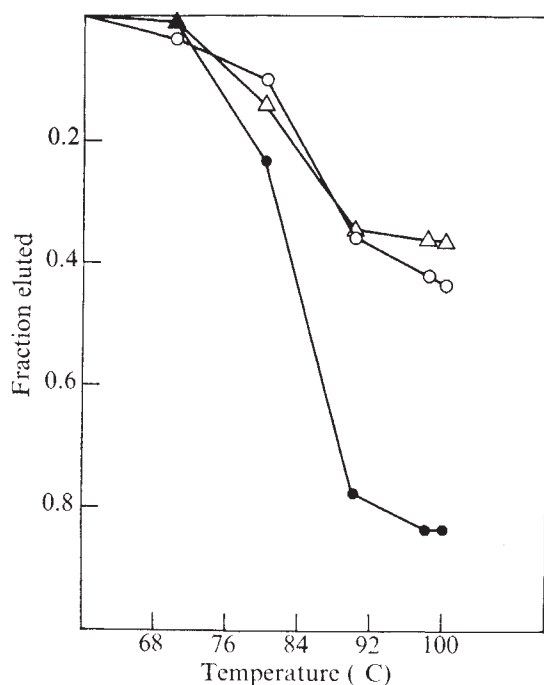


Fig. 3 Competition radioimmunoassays. HL23V virus was assayed for the presence of proteins antigenically related to the p30 (a) and gp45 (b) of NC-37 grown SSV-1. Antisera used for the studies with SSV-p30 were prepared by immunising rabbits with the purified p30. An NC-37 absorbed rabbit antiserum prepared by inoculation of disrupted virus and used for the studies with SSV-gp45 was kindly supplied by Dr D. Larson (Pfizer, Maywood, New Jersey). The p30 protein of SSV-1 was purified from sonicated virus, followed by phosphocellulose and Sephadex G75 column chromatography and isoelectric focusing. It was iodinated and repurified as described by Hunter¹⁸. The iodinated p30 antigen preparations obtained had specific activities of 6×10^6 c.p.m. μg^{-1} . The gp45 protein of NC-37 grown SSV was purified from sonicated virus by column chromatography on Agarose 5M guanidium hydrochloride. Analysis of the preparations by 5% sodium dodecyl sulphate polyacrylamide gel electrophoresis and isoelectric focusing indicated homogeneity of the 45,000-dalton antigen. The viral protein was labelled by the procedure of Bolton and Hunter¹⁹. Succinimidyl-(3-4-hydroxyphenyl) proportionate (ICN Pharmaceuticals, Inc.) was first labelled with ^{125}I and purified. The purified gp45 was then labelled by conjugation with the ^{125}I -labelled ester and chromatographed on a Sephadex G-25 column. Titrations of the antisera were performed in 200- μl reactions that included 0.2% bovine serum albumin (BSA) in saline, 5,000 c.p.m. of ^{125}I -antigen and dilutions of antibody. After 1 h incubation at 37 °C, 100 μg of normal rabbit carrier IgG and a titred amount of goat anti-rabbit IgG were added. The reaction was incubated for 15 h at 4 °C. The samples were centrifuged and both precipitates and supernatants were counted in a Searle Autogamma counter model 1185. The results are expressed as percentage c.p.m. precipitated. More than 80% of the labelled antigen could be bound by specific antisera. Competition assays were performed in a similar manner, except that unlabelled competing antigen was added to the original incubation mixture. The unlabelled competing antigens were SSV (○); HL-23 (□); SSV-gp45 (●) and MPMV (△).

BV-M7 and SSV-1 viral RNAs. These data indicate that the genetic information of HL23V virions is accounted for, within the limits of the sensitivity of the molecular hybridisation techniques used. It would seem that the HL23V genetic information contains the genomes of both SSV-1 and the BV-M7.

To supplement and confirm these findings by an independent method, competition molecular hybridisations were performed using cDNA synthesised from SSV. Viral 70S

RNA from SSV was radiolabelled with ^{125}I . SSV cDNA can protect this ^{125}I -SSV RNA more than 79% from ribonuclease digestion at molar input ratios of 5:1 (cDNA-RNA). Table 1 shows that when cold viral and other RNAs were added in vast excess to complete this homologous reaction, both SSV and HL23V RNA rendered the ^{125}I -SSV completely digestible. The GALV-1 competed less successfully, illustrating the sensitivity of this technique for detecting the small sequence differences known to exist between GALV and SSV.

HL23V was further tested for its immunological similarity to SSV and BV-M7. Virus was concentrated by ultracentrifugation from culture supernatants of A204(HL23V) and used as competing antigens in radioimmunoassays for the p30 and gp45 of NC-37 grown SSV. As shown in Fig. 3, the slope and extent of competition of HL23V in both of these radioimmunoassays were indistinguishable from SSV. Immunodiffusion analysis of HL23V with antisera prepared against the major internal structural proteins of the woolly monkey (p30) and baboon viruses (p28) demonstrated lines of identity with BV-M7 and SSV-1 indicating that, by these immunological criteria, HL23V is indistinguishable from a mixture of these two agents.

The association of SSV or BV with human material is not confined to the one patient from which HL23V was presumably derived. Four groups have now reported the isolation of agents related to SSV^{1,10-12} from a variety of normal and neoplastic human tissues. Further, in 7 out of 40 cases of acute myelogenous leukaemia, where a decision could be made, an enzyme was found^{13,14} that was neutralised by anti-SSV reverse transcriptase. Fresh¹⁵ or short term cultures¹⁶ of human leukaemic cells have been shown to contain particles capable of generating cDNA having significant homology to SSV. Sherr and Todaro¹⁷ have reported that two human tumours of 33 examined by radioimmunoassay demonstrated BV-M7 p28. The repeated encounters with SSV or BV-related material in human samples suggest that these viruses may be more widespread in the human population than had been suspected.

The fact that the HL23V particles have been identified as known animal viruses does not of itself eliminate them as candidate aetiological agents of the human disease. At the same time, it must be accepted that the identification of unknown tissue culture viruses with known laboratory agents raises the obvious possibility that they arose by contamination.

The key question is of course the relationship of the HL23V particles to human leukaemia. A resolution of this issue with respect to any candidate human tumour virus growing in culture requires answers to the following two questions: (1) Can one provide evidence at the level of protein and/or nucleotide sequence for the presence of the putative agent in the original tumour material from which the tissue culture was established? (2) Can one provide evidence at the level of protein and/or nucleotide sequence for the presence of the putative agent in the tumour cells of other patients with the same disease? Finding evidence for the particles in the original tumour cells would serve to eliminate the trivial explanation that they arose in the culture through laboratory contamination. The answer to the second question will decide the general relevance of the observation to human leukaemia. Unless a positive response is obtained in a large proportion of the patients examined, no basis exists for identifying the HL23V particles as clinically significant aetiological agents of human leukaemia.

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EMERSON CHAN
WILLIAM P. PETERS
RAYMOND W. SWEET

TSUNEYA OHNO
DONALD W. KUFÉ
SOL SPIEGELMAN

*Institute of Cancer Research,
Columbia University,
99 Fort Washington Avenue,
New York, New York 10032*

ROBERT C. GALLO
ROBERT E. GALLAGHER

*National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014*

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- ¹ Gallagher, R. E., and Gallo, R. C., *Science*, **187**, 350-353 (1975).
- ² Teich, N. M., et al., *Nature*, **256**, 551-555 (1975).
- ³ Benveniste, R. E., Lieber, M. M., Livingston, D. M., Sherr, C. J., and Todaro, G. J., *Nature*, **248**, 17-20 (1974).
- ⁴ Kalter, S. S., et al., *Science*, **179**, 1332-1333 (1973).
- ⁵ Theilen, G. H., Gould, D., Fowler, M., and Dungworth, D. L., *J. natn. Cancer Inst.*, **47**, 881-889 (1971).
- ⁶ Okabe, H., et al., *Nature*, **260**, 264-266 (1976).
- ⁷ Schlom, J., and Spiegelman, S., *Science*, **174**, 840-843 (1971).
- ⁸ Comerford, S. L., *Biochemistry*, **10**, 1993-2000 (1971).
- ⁹ Tereba, A., and McCarthy, B. J., *Biochemistry*, **12**, 4675-4679 (1973).
- ¹⁰ Panem, S., Prochownik, E. V., Reale, F. R., and Kirsten, W. H., *Science*, **189**, 297-299 (1975).
- ¹¹ Nooter, K., Aarssen, A. M., Bentvelzen, P., de Groot, F. G., and van Pelt, F. G., *Nature*, **256**, 595-597 (1975).
- ¹² Gabelman, G., Waxman, S., Smith, W., and Douglas, S. D., *Int. J. Cancer*, **16**, 355-369 (1975).
- ¹³ Todaro, G. J., and Gallo, R. C., *Nature*, **244**, 206-209 (1973).
- ¹⁴ Gallo, R. C., et al., *Cold Spring Harb. Symp. quant. Biol.*, **39**, 933-961 (1974).
- ¹⁵ Gallo, R. C., Miller, N. R., Saxinger, W. C., and Gillespie, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3219-3224 (1973).
- ¹⁶ Mak, T. W., Kurtz, S., Manaster, J., and Housman, D., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 623-627 (1975).
- ¹⁷ Sherr, C. J., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4703-4707 (1974).
- ¹⁸ Hunter, H. M., in *Handbook of Experimental Immunology*, Ch. 17 (Blackwell, Oxford, 1973).
- ¹⁹ Bolton, A. E., and Hunter, W. M., *Biochem. J.*, **133**, 529-539 (1973).

Construction of a P plasmid carrying nitrogen fixation genes from *Klebsiella pneumoniae*

THE wide host range of plasmids of the P-incompatibility class^{1,2} has attracted interest in this group of R factors. A 3×10^6 -dalton sequence which includes the TEM β -lactamase gene found on some plasmids of this group can integrate into the *Escherichia coli* chromosome³ and into plasmids of other compatibility groups^{4,5}. This translocatable sequence has been termed transposon A (TnA)^{4,6} and resembles the insertion sequences (IS) of *E. coli*, in that TnA insertions can result in gene inactivation, giving rise to strong polar mutations⁷.

We have described⁸ the construction of an F-prime factor, FN68, carrying the nitrogen fixation (*nif*) and *his* region of the *Klebsiella pneumoniae* chromosome; other chromosomal markers detected on this plasmid include *metG*, *gnd* and *shlA*, though it is uncertain whether these determinants originated from *Klebsiella* or from *E. coli*. FN68 carries a TnA sequence which was translocated from R68, a plasmid of the P-incompatibility group. We have observed that FN68 can interact with the P plasmid RP4, which also possesses the TnA sequence, resulting in the formation of a recombinant molecule which retains P-incompatibility properties and carries the *nif his* region of the *K. pneumoniae* chromosome.

The P-group R factor RP4, which determines resistance to kanamycin (Km^R), tetracycline (Tc^R) and carbenicillin (Carb^R) was introduced into an *E. coli* K12 strain SB1801, carrying FN68. The resultant strain SB1801 (RP4) (FN68) was mated in broth with a His^- recipient *E. coli* UNF502 (Table 1, Experiment 1). F-prime transfer (selection for *his*) occurred at a higher frequency than R-factor transfer (selection for Km^R) but cotransfer of both markers was not infrequent. Ten of the

Table 1 Transfer properties of *nif* plasmids in *E. coli* K12 strains

Exp. no.	Donor	Recipient	Selection	Mating	Transfer frequency	Exconjugant phenotype
1	SB1801 (FN68) (RP4)	UNF502	His ⁺ Km ^R	B B	3 × 10 ⁻² 7 × 10 ⁻⁵	
2	JC5466 (RP41)	AB2463	His ⁺ Km ^R Km ^R His ⁺	B S S	3 × 10 ⁻⁶ 5 × 10 ⁻¹ 5 × 10 ⁻¹	10/20 His ⁺ Nif ⁺ Carb ^R Tc ^R Km ^R MS2 ^R PRR1 ^S 29/29 Carb ^R Tc ^R His ⁺ Nif ⁺ 28/29 Km ^R Carb ^R Tc ^R Nif ⁺ 1/29 Km ^R Carb ^R Tc ^R Nif ⁺
3	JC5466 (RP41)	SB1801	Km ^R His ⁺	S S	5 × 10 ⁻¹ 5 × 10 ⁻¹	10/10 Carb ^R Tc ^R His ⁺ Nif ⁺ 10/10 Carb ^R Tc ^R Km ^R Nif ⁺
4	JC5466 (RP41)	SB1801	Km ^R His ⁺	B B	2 × 10 ⁻³ 2 × 10 ⁻³	10/10 Carb ^R Tc ^R His ⁺ Nif ⁺ 10/10 Carb ^R Tc ^R Km ^R Nif ⁺

Donors in broth matings (B) were late log-phase cultures grown in nutrient broth with slow shaking at 37 °C and contained about 2 × 10⁸ bacteria per ml. Recipients were grown overnight in nutrient broth without shaking. For agar surface matings (S), 0.1 ml of a late-log donor culture was mixed with 0.1 ml of a late-log recipient culture and the mixture plated on nutrient agar for 6 h at 37 °C. Bacteria were scraped off the surface of the plates, resuspended in saline phosphate buffer and plated on selective media. Transfer frequencies were calculated relative to the number of donors in the mating mixture at the time of plating on selective media. Transfer frequencies determined for surface matings can only be approximate since differential growth of donor and recipient occurred during the prolonged incubation of mating mixtures on agar medium. *E. coli* K12 strains were:

SB1801, *his*-750 (deletion covering *his gnd* RHA-2A) *str*^R *λ*^R *ara*⁻ *gal*⁻ *malA*⁻ *xyl*⁻ *mtl*⁻ (*λ*⁻);

AB2463, *thi*⁻ *arg*⁻ *his*-4 *pro*⁻ *thr*⁻ *leu*⁻ *str*^R *recA*13 *lac*⁻ *gal*⁻ *ara*⁻ *xyl*⁻ *mtl*⁻;

UNF502, *thi*⁻ *arg*⁻ *his*-4 *pro*⁻ *thr*⁻ *leu*⁻ *str*^R *lac*⁻ *gal*⁻ *ara*⁻ *xyl*⁻ *mtl*⁻ *nal*^R;

JC5466, *trp*⁻ *recA*56 *his*⁻ *spc*^R.

Plasmids were:

FN68, F-incompatibility group F' *metG*⁺ *gnd*⁺ *his*⁺ *nif*⁺ *shiA*⁺;

RP4, P-incompatibility group Km^R Carb^R Tc^R;

RP41, recombinant plasmid described in the text.

Km^R, Resistance to kanamycin sulphate, 30 µg ml⁻¹; Tc^R, resistance to tetracycline-HCl, 15 µg ml⁻¹; Carb^R, resistance to carbenicillin (Beechams), 200 µg ml⁻¹.

twenty His⁺ Km^R exconjugants obtained were Carb^R Tc^R Nif⁺ and sensitive to P-specific phage PRR1, but had lost sensitivity to F-specific phage MS2. Several of these clones cotransferred *his nif* and Km^R to the *E. coli recA* strain JC5466. One derivative of JC5466 was studied in detail; it contained a plasmid designated RP41 which cotransferred Km^R Carb^R Tc^R *his* and *nif* at high frequency in matings carried out on an agar surface (Table 1, Experiments 2 and 3) but at low frequency in broth matings (Table 1, Experiment 4). Such differences between surface and liquid matings are characteristic of P plasmids⁸.

RP41 did not coexist with the P-group plasmid, R751; when selection was made for trimethoprim resistance (a marker on R751), 35 exconjugants tested were His⁻ Nif⁻ and sensitive to tetracycline, kanamycin and carbenicillin (Table 2, Experiment 3). RP41 and the F-prime factor F'Cm, however, coexisted stably (Table 2, Experiments 4 and 5). These results suggest that RP41 has P-group incompatibility and does not retain the incompatibility properties of F. RP41 was stable in the *E. coli recA* host JC5466 and no loss of *his nif* or drug resistance was detected after 15 generations in nutrient broth.

The nitrogenase activity of JC5466 (RP41) as measured by acetylene reduction *in vivo*⁸ was 60 mol ethylene per mg protein per min, compared with 63 mol ethylene per mg protein per min for *K. pneumoniae* strain HF3 which has chromosomally located *nif* genes; both strains having been assayed in identical conditions. The host strain JC5466 did not reduce acetylene. In addition to *his* and *nif* genes, RP41 also probably carries a determinant for gluconate-6-phosphate dehydrogenase (*gnd*) since a *gnd* deletion strain *E. coli* SB1801 gave a positive reaction in the colorimetric assay for this

enzyme⁹ when RP41 was present. When RP41 was transferred to AB 2880, a His⁻ ShiA⁻ AroD⁻ recipient, His⁺ exconjugants inherited *shiA*, a marker located close to *his*, but not *aroD*. In contrast to the F'*nif*, FN68, RP41 does not carry *metG*, since cotransfer of this determinant with *his* was not detected in a cross with a His⁻ MetG⁻ derivative of *E. coli* C, and 30 His⁺ exconjugants derived from this cross did not grow in the absence of methionine. RP41 thus carries the determinants *gnd his nif shiA* derived from FN68, but lacks *metG*.

Covalently closed-circular (CCC) DNA isolated from *E. coli* strain J62-1 carrying RP41 sedimented at 72S in neutral sucrose and 171S in alkaline sucrose gradients (Fig. 1) corresponding to a molecular weight of 59 × 10⁶.

RP41 was transferable from *E. coli* to *K. pneumoniae* at high frequency. When the recipient was *K. pneumoniae* Kp52, a *nif his* deletion strain, no segregation of plasmid markers was detectable (Table 3, Experiment 1). Drug resistance, *his* and *nif* were cotransferred from *K. pneumoniae* Kp52 (RP41) to *E. coli* at high frequency (Table 3, Experiment 2).

It was also possible to transfer RP41 to a His⁻ derivative of the unrelated bacterium *Agrobacterium tumefaciens*; co-transfer of *his* and drug resistance was observed but 10 clones tested were Nif⁻ and did not give acetylene reduction in either anaerobic or aerobic conditions (Table 3, Experiment 3). Crude extracts of one of the His⁺ exconjugants grown with low aeration in nitrogen-free medium containing aspartate (100 µg ml⁻¹), however, produced material which cross reacted antigenically (CRM) with antiserum prepared against purified *K. pneumoniae* nitrogenase molybdoprotein. No CRM was detected in extracts prepared from cultures grown on NH₄⁺

Table 2 Compatibility properties of RP41

Exp. no.	Donor	Recipient	Selection	Transfer frequency	Exconjugant phenotype
1	J62-2 (R751)*	SB1801	Trim	> 1	
2	J62-2 (R751)*	SB1801 (RP4)	Trim	2 × 10 ⁻⁴	
3	J62-2 (R751)*	SB1801 (RP41)	Trim	1 × 10 ⁻⁴	35/35 Trim ^R Carb ^R Tc ^R Km ^R His ⁻ Nif ⁻
4	F'Cm†	SB1801	Cm	7 × 10 ⁻¹	
5	F'Cm†	SB1801 (RP41)	Cm	2 × 10 ⁻¹	36/36 Cm ^R Carb ^R His ⁺ Nif ⁺ Tc ^R Km ^R

Matings were carried out on an agar surface* or in broth† as described in the legend to Table 1. *E. coli* K12 strain: J62-2 *pro*⁻ *his*⁻ *trp*⁻ *lac*⁻ *spc*^R. Plasmids: F Cm, F prime factor, determines chloramphenicol resistance, (Cm^R) 30 µg ml⁻¹, R751, P-group plasmid, determines resistance to trimethoprim lactate (Trim), 50 µg ml⁻¹. All other bacterial strains, plasmids and symbols are as in Table 1.

as 1 mg $(\text{NH}_4)_2\text{SO}_4$ per ml, a nitrogen source which represses nitrogenase synthesis in *Klebsiella*. When RP41 was transferred back to *E. coli*, several exconjugants were Nif^+ , confirming that *K. pneumoniae nif* genes had been transferred to *Agrobacterium* (Table 3, Experiment 4).

Transfer of RP41 to *Rhizobium meliloti* occurred at low frequency and some segregation of plasmid markers was observed; only 2 out of 10 His^+ exconjugants were tetracycline resistant and only 12 of 20 Tc^R exconjugants were His^+ (Table 3, Experiment 5). RP41 was unstable in *R. meliloti*; a 48% segregation of *his* was observed after 3 subcultures in L broth. Ten His^+ and twenty Tc^R exconjugants did not reduce acetylene when tested in either aerobic or anaerobic conditions. A faint band of cross-reacting material for *Klebsiella* nitrogenase molybdoprotein was observed in extracts prepared from cultures grown on nitrogen-free medium containing aspartate but not from NH_4^+ -repressed cultures. *Nif* genes were expressed when the plasmid was transferred back to *E. coli*, indicating that *K. pneumoniae nif* genes were present in *R. meliloti* (Table 3, Experiment 6).

Transfer of RP41 to *Pseudomonas aeruginosa* was not observed. Two *his*⁻ strains known to accept RP4 (strains PA067 and PAT904) were tested by surface and liquid matings and no His^+ or Carb^R derivatives appeared.

Although the precise mechanism of the recombinational events described here remains obscure, it is probable that cointegration was promoted by insertion sequences associated with the β -lactamase genes present in both parent plasmids. We suggest that cointegration of FN68 and RP4 resulted in excision of a segment of FN68 DNA which included F sex factor genes and *metG*, by a process analogous to that shown for other plasmids formed *in vivo* by site-specific recombination¹⁰. RP41 may resemble a similar plasmid, RP1-*his* described by Olsen and Gonzalez¹¹. Both plasmids show stable linkage of drug-resistance genes and *his* in *E. coli* but both plasmids dissociate in intergeneric matings.

A P plasmid carrying nitrogen fixation genes has many advantages over an F'*nif* factor for genetic studies of nitrogen fixation. It transfers at high frequency from *E. coli* to *K. pneumoniae* and retains transferrability in this host in contrast to the F'*nif* FN68 which is *Tra*⁻ in *Klebsiella*⁸. Mutants of RP41 could therefore be useful for complementation analysis of the *K. pneumoniae nif* region. RP41 also has a wide host range and should be transmissible to many Gram-negative

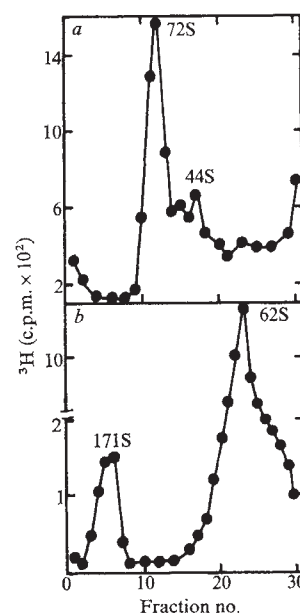


Fig. 1 Profiles of ^3H -labelled DNA from *E. coli* J62-1 (RP41) analysed by velocity centrifugation in neutral (a) and alkaline (b) 5 ml, 5–20% sucrose gradients. Supercoiled DNA, obtained from ethidium bromide–CsCl gradients⁸, was dialysed against TES buffer (10 mM Tris-HCl, 1 mM Na_2EDTA , 100 mM NaCl pH 8.0). RP41 DNA (180 μl) was mixed with 20 μl of ^{14}C -labelled Cole1 DNA, layered on gradients and centrifuged for 60 min (a) and 33 min (b) at 120,000g at 20°C in a 52 rotor using a Christ ωII ultracentrifuge. Gradients were fractionated by drop collection (7 drops per fraction) directly on to filter disks and TCA-precipitable label assayed for radioactivity.

bacteria, although the failure to obtain transfer to *Pseudomonas aeruginosa* suggests that the host range has been modified.

The failure to express *K. pneumoniae nif* genes in *R. meliloti* is perhaps not surprising, since *R. meliloti* itself has *nif* genes which were not expressed in the conditions tested, but which are expressed in legume root nodules. It will be interesting to observe whether the *K. pneumoniae nif* system can be expressed in *R. meliloti* in symbiotic conditions or in the conditions developed for expression of nitrogenase in certain free-living rhizobia^{12–16}. We did find CRM to antiserum to *Klebsiella* nitrogenase molybdoprotein in extracts of derepressed cultures

Table 3 Intergeneric matings with RP41

Exp. no.	Donor	Recipient	Selection	Transfer frequency	Exconjugant phenotype
1	<i>E. coli</i> K12 JC5466 (RP41)	<i>K. pneumoniae</i> M5a1 Kp52	His^+ Km^R	1×10^{-1} 1×10^{-1}	87/87 $\text{Km}^R\text{Tc}^R\text{Carb}^R\text{Nif}^+$ 56/56 $\text{Tc}^R\text{Carb}^R\text{Nif}^+\text{His}^+$
2	<i>K. pneumoniae</i> M5a1 Kp52 (RP41)	<i>E. coli</i> K12 SB1801	His^+ Km^R	5×10^{-1} 5×10^{-1}	29/29 $\text{Km}^R\text{Tc}^R\text{Carb}^R\text{Nif}^+$ 24/24 $\text{Tc}^R\text{Carb}^R\text{Nif}^+\text{His}^+$
3*	<i>E. coli</i> K12 JC5466 (RP41)	<i>Agrobacterium tumefaciens</i> 544	Tc^R His^+	3×10^{-3} 1×10^{-3}	52/52 Km^RHis^+ 52/52 Km^RTc^R 0/10 Nif^+
4*	<i>A. tumefaciens</i> 544 (RP41)	<i>E. coli</i> K12 JC5466	His^+ Km^R	1×10^{-6} 1×10^{-6}	10/10 $\text{Km}^R\text{Tc}^R\text{Nif}^+$ 10/10 $\text{His}^+\text{Tc}^R\text{Nif}^+$
5†	<i>E. coli</i> K12 JC5466 (RP41)	<i>Rhizobium meliloti</i> A1	His^+ Tc^R	6×10^{-5} 1×10^{-5}	2/10 Tc^R 0/10 Nif^+ 12/20 His^+ 0/20 Nif^+
6†	<i>R. meliloti</i> A1 (RP41)	<i>E. coli</i> JC5466	His^+ His^+Nif^+ Km^R	3×10^{-2} 3×10^{-2} 1×10^{-2}	21/41 Km^R 19/20 Nif^+ 28/33 His^+

Crosses were carried out on an agar surface as described in Table 1.

*Mating mixtures were prepared on plate count agar (Difco) and were incubated for 16 h before plating on selection media. Transfer frequencies were calculated relative to the number of recipients in the mating mixture before plating on the selection media.

†Mating mixtures were prepared on L agar and incubated for 16 h before exconjugant selection.

Strains: *Klebsiella pneumoniae* Kp52, (deletion covering *nif his* and *gnd*) P2 eductant of M5a1; *Agrobacterium tumefaciens* 544 *his*⁻ Sm^R ; *Rhizobium meliloti* A1 *his*⁻ *bio*⁻ (derivative of *R. meliloti* 41).

of both *Rhizobium* and *Agrobacterium*, suggesting that at least one *nif* gene from *K. pneumoniae* can be transcribed and translated in these bacteria.

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RAY DIXON
FRANK CANNON

ARC Unit of Nitrogen Fixation,
University of Sussex,
Brighton BN1 9QJ, UK

ADAM KONDOROSI

Institute of Genetics,
Biological Research Centre,
Hungarian Academy of Sciences,
H-6701 Szeged pf. 521, Hungary

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- 1 Datta, N., Hedges, R. W., Shaw, E. J., Sykes, R. B., and Richmond, M. H., *J. Bact.*, **108**, 1244–1249 (1971).
- 2 Olsen, R. H., and Shipley, P., *J. Bact.*, **113**, 772–780 (1973).
- 3 Sykes, R. B., and Richmond, M. H., *Nature*, **226**, 952–954 (1970).
- 4 Hedges, R. W., and Jacob, A. E., *Molec. gen. Genet.*, **132**, 31–40 (1974).
- 5 Beringer, J. E., *Heredity*, **33**, 134 (1974).
- 6 Heffron, F., Sublett, R., Hedges, R. W., Jacob, A., and Falkow, S., *J. Bact.*, **122**, 250–256 (1975).
- 7 Heffron, F., Rubens, C., and Falkow, S., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3623–3627 (1975).
- 8 Cannon, F. C., Dixon, R. A., and Postgate, J. R., *J. gen. Microbiol.*, **93**, 111–125 (1976).
- 9 Peyru, G., and Fraenkel, D. G., *J. Bact.*, **95**, 1272–1278 (1968).
- 10 Kopecko, D. J., and Cohen, S. N., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1373–1377 (1975).
- 11 Olsen, R. H., and Gonzalez, C., *Biochem. biophys. Res. Commun.*, **59**, 377–385 (1974).
- 12 Pagan, J. D., Child, J. T., Scowcroft, W. R., and Gibson, A. H., *Nature*, **256**, 406–407 (1975).
- 13 Kurz, W. G. W., and LaRue, T. A., *Nature*, **256**, 407–408 (1975).
- 14 McComb, J. A., Eliot, J., and Dilworth, M. J., *Nature*, **256**, 409–410.
- 15 Tjepkema, J., and Evans, H., *Biochem. biophys. Res. Commun.*, **65**, 625–627 (1975).
- 16 Keister, D. L., *J. Bact.*, **123**, 1265–1267 (1975).

Expression of *Klebsiella* nitrogen fixation genes (*nif*) in *Azotobacter*

NITROGEN fixation mutants have been isolated and biochemically characterised in *Azotobacter vinelandii*¹, but the absence of chromosomal gene transfer systems such as conjugation in this genus has prevented a genetic analysis of such mutants. We now report the intergeneric transfer of

Klebsiella nif genes carried on the P-type plasmid, RP41 (refs 2 and 3), to *Azotobacter*, and their expression in this host.

The *A. vinelandii nif* mutants lacked nitrogenase molybdo-iron protein (AvI) activity, iron protein (AvII) activity or were pleiotropically negative for both activities. Spontaneous derivatives resistant to 25 µg ml⁻¹ nalidixic acid were isolated before mating with an *Escherichia coli* K12 strain carrying the *nif* plasmid RP41. Matings were done on agar plates: cultures of donor *E. coli* grown at 37 °C in Oxoid nutrient broth and *A. vinelandii* recipients grown at 28 °C in Burk's sucrose medium⁴ supplemented with 2 mg ml⁻¹ ammonium acetate were grown to mid logarithmic phase and mixed in a 1 : 1 ratio; 0.1-ml portions of mixtures were spread on Oxoid nutrient agar plates and incubated overnight at 34 °C. Mating mixtures and parental *azotobacter* cultures were resuspended and diluted in sucrose-free Burk's medium⁵ and spread on selective agar media: Burk's sucrose medium containing 25 µg ml⁻¹ nalidixic acid to select Nif⁺ exconjugants and a similar medium with 2 mg ammonium acetate and 10 µg ml⁻¹ tetracycline when tetracycline-resistant exconjugants were sought. Selective plates were incubated at 28 °C; all incubations were in air.

Presumptive nitrogen-fixing exconjugants were tested for acetylene-reducing ability by transfer to 5 ml liquid Burk's sucrose medium in 25-ml conical flasks. After growth at 30 °C, the cotton wool plugs were replaced by Suba-seal rubber closures. 2 ml C₂H₂ was injected and gas samples were removed at intervals for gas chromatography on a Porapak N column with a flame ionisation detector. Activities were compared with that of wild-type *A. vinelandii* grown in similar conditions.

Among the markers carried by RP41 are *nif*, *his*, resistance to tetracycline (Tc), kanamycin (Km) and carbenicillin (Carb). The transfer frequencies of *nif* and Tc to *Azotobacter* mutants are shown in Table 1. In contrast to the situation in *Klebsiella* and *E. coli*, Nif⁺ exconjugants could be selected directly on plates in air. Although the transfer frequencies were low, they were significantly higher than reversion frequencies. The low observed frequencies arose at least partially from the poor growth of *A. vinelandii* on the mating plates: ~10⁷ *A. vinelandii* compared with ~10⁹ *E. coli* ml⁻¹ when resuspended at the end of the mating period.

Fifteen of the 83 Nif⁺ exconjugants listed in Table 1, including representatives of all positive recipients, were tested for nitrogenase by the acetylene test and all showed activity between 40% and 50% of that of the wild type.

Table 1 shows that nitrogenase activity was restored to three mutants which lacked AvI and one which lacked AvII activity but not to the one strain examined (UW1) which

Table 1 Intergeneric transfer of plasmid RP41 from *E. coli* K12 to *A. vinelandii*

Recipient strains	Nitrogenase activity*	Frequency of reversion to Nif ⁺ †	Selection§	Transfer frequency‡	Exconjugant phenotype§		
UW1	I ⁻ II ⁻	< 4 × 10 ⁻⁸	Tc ^R Nif ⁺	3.8 × 10 ⁻⁷ < 10 ⁻⁷	28/28Km ^R , —	27/28Carb ^R , —	0/28Nif ⁺
UW10	I ⁻ II ⁺	9 × 10 ⁻⁸	Tc ^R Nif ⁺	2.0 × 10 ⁻⁶ 5.4 × 10 ⁻⁷	27/28Km ^R , 17/17Tc ^R ,	24/26Carb ^R , 17/17Carb ^R ,	25/26Nif ⁺ , 13/17Km ^R
UW100	I ⁻ II ⁺	< 5 × 10 ⁻⁸	Tc ^R Nif ⁺	4.0 × 10 ⁻⁷ 1.3 × 10 ⁻⁶	18/18Tc ^R ,	0/9 Carb ^R , 18/18Carb ^R	9/9Nif ⁺
UW38	I ⁻ II ⁺	< 2 × 10 ⁻⁸	Tc ^R Nif ⁺	1.4 × 10 ⁻⁷ 3.8 × 10 ⁻⁶	21/28Tc ^R ,	27/28Carb ^R , 18/28Carb ^R	27/28Nif ⁺
UW91	I ⁺ II ⁻	< 3 × 10 ⁻⁸	Tc ^R Nif ⁺	9.0 × 10 ⁻⁷ 4.1 × 10 ⁻⁷	19/19Tc ^R	—	22/28Nif ⁺

*W. J. Brill, personal communication (I and II are the molybdo-iron protein and iron protein, respectively, of nitrogenase).

†Reversion frequencies determined as for transfer frequencies but without donor cells. Minima signify no revertants detected with the population tested.

‡Transfer frequencies determined after plate matings (carried out as described in text) and expressed relative to the numbers of donor bacteria *E. coli* JC5466(RP41), at the end of the mating period.

§Symbols: Tc^R, resistance to 10 µg tetracycline; Km^R, resistance to 20 µg kanamycin; Carb^R, resistance to 200 µg carbenicillin ml⁻¹.

Table 2 Transfer of RP41 from *A. vinelandii* to *E. coli* K12

<i>A. vinelandii</i> donor strains	<i>E. coli</i> recipients	Selection	Transfer frequency	Exconjugant phenotype
UW100(RP41)	JC5466	His ⁺ Tc ^R Km ^R	3.2×10^{-7} 2.0×10^{-7} 1.4×10^{-7}	7/8 Km ^R Tc ^R Nif ⁺ 1/7 His ⁺ Nif ⁺ , 7/7 Km ^R 0/9 His ⁺ Nif ⁺ , 9/9 Tc ^R
UW100(RP41)	SB1801	His ⁺ Km ^R	3.0×10^{-4} 3.0×10^{-2}	28/28 Nif ⁺ Tc ^R 28/28 Tc ^R , 0/28 His ⁺ Nif ⁺
UW91(RP41)	SB1801	His ⁺ Km ^R	5.0×10^{-6} 1.0×10^{-5}	8/8 Km ^R Nif ⁺ 8/8 Tc ^R , 0/8 Nif ⁺ His ⁺

Plate matings as described in text. Genotype of JC5466 is *trp his recA56 spc*; Genotype of SB1801 is *his750* (deletion extending through *his gnd RHA-2A*) *str* λ^R *ara gal mala xyl mtl* (λ^-). Symbols as in legend to Table 1.

was pleiotropically negative for both component activities. The presence of RP41 *nif* genes in one of each class of Nif⁺ exconjugants, UW91(RP41) and UW100(RP41), was confirmed by using these strains as donors and observing transfer of *his*, *nif* and drug resistance markers back to *E. coli* (Table 2). Some segregation of markers occurred in these matings: exconjugants selected directly for drug resistance were mainly His⁻ and Nif⁻ while all but one of the His⁺ exconjugants were Nif⁺ and drug resistant. Such segregation has been observed in other transfers involving *nif* plasmids^{3,6}.

Loss of RP41 *nif* genes ought to restore the Nif⁻ phenotype in these exconjugants. One *Azotobacter* strain UW91 (RP41) was cultured in non-selective conditions and spontaneous loss of *nif* genes occurred: After 10 subcultures in Burk's medium supplemented with 2 mg ml⁻¹ ammonium acetate, all clones among 52 tested were Nif⁻ and 28, screened for drug resistance, were resistant to Tc, Km and Carb. These drug-resistance markers, which originated on RP4, the parent plasmid of RP41 (ref. 2), were stable in *A. vinelandii*.

One *Azotobacter* strain, UW100(RP41) was examined for immunological evidence for the expression of *Klebsiella nif* structural genes. Crude extracts of this strain, grown in Burk's medium, contained material which cross reacted antigenetically with antiserum prepared against purified *K. pneumoniae* nitrogenase molybdoprotein (KpI). This antigen was absent from extracts of the parental strain, UW100, grown in ammonium-limited conditions (100 μ g N (as ammonium acetate) ml⁻¹ Burk's medium). Material cross reacting with antiserum to purified *Azotobacter* nitrogenase molybdoprotein (AvI) was, however, present in both extracts. When these strains were grown with excess NH₄⁺ ion, no such cross-reacting materials were detected in extracts prepared similarly.

We conclude from these experiments that the Nif⁺ *Azotobacter* exconjugants carried RP41 and expressed its *nif* genes, which originated in *Klebsiella pneumoniae* strain M5a1 (ref. 2). The ability of *Azotobacter* to transcribe and translate *Klebsiella nif* genes and, in contrast to *Agrobacterium*³, actually to make use of their products has several important consequences:

(1) The regulatory apparatus, whereby ammonia repressed nitrogenase synthesis in parents and exconjugants, is common to *Klebsiella* and *Azotobacter*, even at a molecular level. For example, if glutamine synthetase (GS) is accepted as a positive activator of *nif* genes⁷⁻⁹, then *Azotobacter* GS can activate *Klebsiella nif* genes.

(2) *Klebsiella nif* genes normally function only in an anaerobic environment, yet transfer to the obligate aerobe *Azotobacter* permitted their expression in air. Clearly they must have shared the oxygen exclusion processes¹⁰ of *Azotobacter*.

(3) The possibility arises that hybrid nitrogenases, such as the active mixtures of AcI+KpII or KpI+AcII which can be studied *in vitro*¹¹, are functioning *in vivo*, but comparable experiments with characterised mutants of RP41 would be necessary to establish this point conclusively.

RP41 is thus potentially useful for a genetic complementation analysis of *nif* mutants in *Azotobacter*. It could also be used for the isolation of selected mutants in *Azotobacter* by introducing RP41 with characterised *nif* mutations into *Azotobacter* and preparing homogenates.

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F. C. CANNON
J. R. POSTGATE

ARC Unit of Nitrogen Fixation,
University of Sussex,
Brighton BN1 9QJ, UK

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- Shah, V. K., Davis, L. C., Gordon, J., Orme-Johnson, W. H., and Brill, W. J., *Biochim. biophys. Acta*, **292**, 246 (1973).
- Dixon, R. A., Cannon, F. C., and Kondorosi, A., *Proc. Soc. gen. Microbiol.*, **2**, 43 (1975).
- Dixon, R. A., Cannon, F. C., and Kondorosi, A., *Nature*, **260**, 268-271 (1976).
- Newton, J. W., Wilson, P. W., and Burris, R. H., *J. biol. Chem.*, **204**, 445 (1953).
- Billon, S., Williams, K., and Postgate, J. R., *J. appl. Bact.*, **33**, 270 (1970).
- Cannon, F. C., Dixon, R. A., and Postgate, J. R., *J. gen. Microbiol.*, **93**, 111 (1976).
- Gordon, J., and Brill, W. J., *Biochem. biophys. Res. Commun.*, **59**, 967 (1974).
- Stretcher, S., Shanmugam, K., Ausubel, F., Morandi, C., and Goldberg, R., *J. Bact.*, **120**, 815 (1974).
- Tubb, R. S., *Nature*, **251**, 481 (1974).
- Postgate, J. R., *The Biology of Nitrogen Fixation* (edit. by Quispel, A.), 663 (North Holland, Amsterdam, 1974).
- Thorneley, R. N. F., Eady, R. R., and Yates, M. G., *Biochim. biophys. Acta*, **403**, 269 (1975).

Errata

In the article "Cell differentiation by 3',5'-cyclic AMP in a lower plant" by A. K. Handa and M. M. Johri (*Nature*, **259**, 480; 1976) there is an error in Table 3. The entries under the heading 'Medium' should read . . . MM, MM+1% glucose, MM+1 μ M IAA, MM+1% glucose . . . and not as printed.

In the paper "Dopamine-like renal and mesenteric vasodilation caused by apomorphine, 6-propylnorapomorphine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene", by H. J. Crumley, R. M. Pinder, W. B. Hinshaw and L. I. Goldberg (*Nature*, **259**, 584; 1976) Dr Pinder's address should be: . . . Birkenhead, Auckland, New Zealand.

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